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### Report to the Great Lakes Research Advisory Board of the International Joint Commission on the Ecological Effects of Non- Phosphate Detergent Builders: Final Report on NTA

International Joint Commission. Task Force on Ecological Effects of Non-Phosphate Detergent  
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# **GREAT LAKES**

## **RESEARCH ADVISORY BOARD**

GLC 22222 129



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**INTERNATIONAL  
JOINT  
COMMISSION**

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**ECOLOGICAL EFFECTS OF  
NON-PHOSPHATE DETERGENT BUILDERS:  
FINAL REPORT ON NTA**







December 1978

**REPORT TO THE**

**GREAT LAKES RESEARCH  
ADVISORY  
BOARD**

**OF THE  
INTERNATIONAL JOINT  
COMMISSION**

**ON THE**

**ECOLOGICAL EFFECTS  
OF NON-PHOSPHATE  
DETERGENT BUILDERS:  
FINAL REPORT ON NTA**

**FROM THE**

**TASK FORCE ON  
ECOLOGICAL EFFECTS  
OF NON-PHOSPHATE  
DETERGENT BUILDERS**

The Research Advisory Board  
effective November 22, 1978  
became the Science Advisory Board.

**DECEMBER, 1978**



REPORT TO THE

# GREAT LAKES RESEARCH ADVISORY BOARD

## NOTICE

Statements and views presented in this report are those of the Task Force members and do not necessarily reflect the views and policies of the International Joint Commission or the Great Lakes Research Advisory Board.

OF THE  
INTERNATIONAL JOINT  
COMMISSION

ON THE

ECOLOGICAL EFFECTS  
OF NON-PHOSPHATE  
DETERGENT BUILDERS:

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INTERNATIONAL JOINT COMMISSION  
GREAT LAKES SCIENCE ADVISORY BOARD



December 1978

Great Lakes Science Advisory Board

International Joint Commission

Canada and the United States

Members of the Board:

The Task Force on Ecological Effects of Non-Phosphate Detergent Builders in partial fulfillment of its responsibilities for meeting its Terms of Reference, hereby submits its final report on NTA. The report is a documentation of the findings presented in the Research Advisory Board's 1977 Annual Report to the International Joint Commission. Reports on other detergent builders currently in use or proposed for use will be presented at a later date.

Respectfully submitted,

Joseph Shapiro (Chairman)

Peter J. Chapman

Richard I. Dick

Peter J. Dillon

Charles R. O'Melia

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OSCAR JACOB

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JOSEPH SHAPIRO (Chairman)

Respectfully submitted,

bulletins currently in use or proposed for use will be presented at a later date. Report to the International Joint Commission. Reports on other developments of the findings presented in the Research Advisory Board's 1977 Annual Reference, hereby submitted as final report on W.A. The Report is a document in partial fulfillment of the responsibility for meeting the needs of the fishery force on knowledge, effects of the fishery's development bulletins.

Members of the Board:

Canada and the United States

International Joint Commission

Great Lakes Science Advisory Board

December 1979

GREAT LAKES SCIENCE ADVISORY BOARD  
INTERNATIONAL JOINT COMMISSION



## ACKNOWLEDGEMENTS

The Task Force is indebted to the Soap and Detergent Associations of Canada and the United States for their assistance in providing a mechanism through which companies interested in NTA could coordinate their efforts to provide information to the Task Force; and to the governments of Canada and the United States for the assistance of their research personnel.

Particular thanks are due to D. R. Rosenberger of the International Joint Commission's Great Lakes Regional Office; F. A. Brownridge of Procter and Gamble Co. of Canada Limited; F. Kennedy of Continental Oil Company; W. E. Lowe and K. Kaiser of the Department of Fisheries and Environment; and to W. Lacy, W. Fairless and J. Welch of the Environmental Protection Agency.

Appreciation is expressed to the Procter and Gamble Company and the Monsanto Company for their major assistance in preparing technical reviews and responding to the many queries of the Task Force; also to those members of the industry, government and academic community listed in the appendices who contributed through presentations and discussions of relevant information. A special note of thanks is also extended to those persons who provided a critical technical review of the draft report. These are: A. F. Beeton, J. M. Tiedje, F. M. Morel, H. E. Allen, J. W. Arthur, F. Huber, and L. F. Centofanti.



L. E. Campbell.

J. M. Hodge, W. E. Hodge, W. E. Hodge, J. M. Hodge, W. E. Hodge, W. E. Hodge, and

critical technical review of the draft report. These and A. E. Hodge, who contributed through presentation and discussion of relevant information of the industry, government and academic community listed in the appendix and responding to the many queries of the Task Force, also to those members Monahan Company for their major assistance in preparing technical reviews.

Appreciation is expressed to the Project and Gamble Company and the to W. Hodge, W. Hodge and J. M. Hodge of the Environmental Protection Agency, W. E. Hodge and K. Hodge of the Department of Fisheries and Environment, and Gamble Co. of Canada Limited; J. Hodge of Continental Oil Company; Joint Commission's Great Lakes Regional Office; E. A. Hodge of Project.

Particular thanks are due to D. E. Hodge of the International the United States for the assistance of their research personnel.

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## INTRODUCTION

This report of the Research Advisory Board's Task Force on Ecological Effects of Non-Phosphate Detergent Builders presents the Task Force's findings on NTA.

During the last two decades it has become clear that phosphorus contained in domestic wastewater is one of the principal contributors to the increasingly rapid process of eutrophication observed in many inland waters, including the Great Lakes. In recognition of this, the 1972 Great Lakes Water Quality Agreement between Canada and the United States called for the development and implementation of programs and other measures to reduce inputs of phosphorus from municipal sewage. Phosphate-based detergents were identified as one of the main sources of controllable wastewater phosphorus. Restriction on detergent phosphate would increase the use and release of alternative materials developed and being developed by the detergent manufacturers. Therefore, the International Joint Commission's Great Lakes Research Advisory Board created a Task Force in 1977 to investigate the potential ecological effects of these non-phosphate detergent builders. The Task Force was directed to examine the available information on the ecological effects of currently existing or proposed alternatives to phosphate and to report on the ecological acceptability of these builders. The terms of reference given to the Task Force by the Board are appended.

The Task Force was constituted of scientists selected from those technical areas that address known and potential environmental effects that might be anticipated. These technical areas are aquatic chemistry, microbial degradation, wastewater management, biological effects, eutrophication, and environmental modelling. Further, to facilitate improved awareness of unpublished data and ongoing research being conducted by industry and governments, liaison members were solicited from the United States and Canadian Soap and Detergent Associations and appropriate federal agencies within Canada and the United States. The Task Force and liaison members are listed in the appendix.

Seven materials were chosen for examination by the Task Force. These are as follows: the organic polycarboxylate builders - citrate, carboxymethyloxysuccinate (CMOS), carboxymethyltartronate (Builder M), and nitrilotriacetate (NTA); and the inorganic builders - carbonate, silicate, and aluminosilicate (zeolite).

NTA was of particular interest to the Task Force. This material was in limited use in the USA and Canada in 1970. Its use expanded when Canada began on a national scale to limit the phosphorus content of detergents. In December of 1970, the Surgeon General of the United States Public Health Service expressed



concern regarding possible health aspects of NTA, and requested that the major detergent manufacturers in the United States voluntarily discontinue its use pending further evaluation. The Canadian Government continued to allow its use as a detergent builder. Because of this and because another Task Force of the Research Advisory Board was completing an investigation of the health aspects of NTA, this Task Force decided to consider and evaluate the ecological effects of NTA first and separately from the other builders.

The procedure followed by the Task Force in its evaluation of NTA was to request liaison members to arrange presentations by industry and government representatives. Following these presentations, scientists who had conducted NTA research or represented pertinent research disciplines were invited to discuss specific aspects of the properties of NTA. In addition, available literature was examined by the Task Force. Members were assigned sections to write, based on areas of expertise. Sections of the report in draft form were circulated to Task Force members for review and comment. Outside technical review was obtained from government and industry and from selected experts. Those who participated in the discussions are listed in the appendix.

The findings of the Task Force on NTA are presented in the following chapters.



## CONCLUSIONS AND RECOMMENDATIONS

The Task Force concludes that the extensive literature and Canadian experience indicates that the use of NTA does not constitute an obvious environmental hazard, however, a number of important questions remain. The Task Force feels that more work should be done to resolve these questions.

Based on this conclusion, the Task Force recommends that NTA should not be prohibited from use as a detergent builder. The Task Force further recommends that its use be accompanied by studies to address the following questions:

- (1) Under aerobic biological wastewater treatment regimes and in most circumstances in fresh water systems NTA ordinarily degrades rapidly. However, the Canadian monitoring experience did reveal that low concentrations of NTA may be found in the aquatic environment. The Task Force feels that the reasons for which NTA exists at these low concentrations should be investigated.

Whenever any new organic compound is released to the environment, there is concern over its persistence. By recommending that the persistence of NTA be investigated, we are in no way comparing the properties of NTA to such materials as halogenated hydrocarbons. On the contrary, we recognize the clear difference between NTA and such substances. But from the Canadian experience, NTA does appear to persist at low levels and it is important to know whether this is because of the kinetics of microbial activity or because of the presence of non-degradable chelates. For example, low temperatures slow the degradation of NTA preferentially and the acclimation time to attain NTA degradation is greater than for more readily biodegradable wastewater constituents.

- (2) Although NTA degrades rapidly in most aerobic environments, studies on its ability to degrade during anaerobic waste treatment or under anaerobic environmental conditions are inconclusive. The Task Force feels the question needs further study.

Some controversy exists on the ability of NTA to degrade under "anaerobic conditions". Some of this controversy may rest on the question of whether certain systems were indeed anaerobic, but in other cases anaerobic degradation apparently has occurred. Furthermore, it has occurred where the concentration of nitrate was probably too low to serve as an electron acceptor. The situation requires clarification.

- (3) No investigations have been conducted to determine the concentrations of NTA in anaerobically digested sludges. Canadian studies have shown that about one-third of the NTA appears to be lost from solution during primary treatment. Because it may have settled out in the sludge and because sludge is frequently deposited in the sea and on land, investigation is necessary.



Because anaerobic degradation of NTA remains in question, it cannot be counted on and therefore NTA is likely to show up in sludges where some, but not necessarily all, will be degraded aerobically. The extent to which it thus persists and comes in contact with land plants and aquatic organisms needs study.

- (4) Although NTA does degrade in aerobic waste treatment, the Task Force feels that its behavior in physical-chemical treatment processes needs elucidation.

Little, if any information, is available on the removal of NTA by such processes as alum coagulation, lime coagulation, iron coagulation, carbon adsorption, etc. Although such processes are likely to follow aerobic wastewater treatment, their effects on NTA should be known.

- (5) NTA degradation has been studied at intermediate pH values (5-9) and at temperatures in the low to moderate range. The behavior of NTA at low and high environmental pH values and at temperatures expected in thermophilic waste treatment processes should be evaluated.

Many eutrophic softwater lakes, particularly those receiving wastewater effluents, maintain pH values well in excess of 9 for long periods of time, yet most studies of degradation have been done at pH values below 9. Similarly, an increasingly large number of streams and lakes are developing low pH values below 5, not only from acid mine drainage in areas such as West Virginia, but in the northeast United States, as well as in southern Scandinavia, from acid rain.

- (6) Degradation of NTA in marine and estuarine systems appears to be very slow. The Task Force feels that because of the versatility of microorganisms it is likely that NTA will not remain in such systems. Further study is required, however.

Degradation of NTA by marine organisms has been demonstrated, but in estuarine systems there apparently is inhibition of the degradation by freshwater organisms. The possibility of accumulation of NTA in such systems should be further examined.

- (7) NTA does not solubilize and transport heavy metals to an appreciable extent under most circumstances. However, the Task Force feels that its behavior in systems of high inorganic turbidity and/or high metal content needs investigation.

There are specific systems where reaction of NTA with heavy metals and subsequent transport of the metals might be especially important. Among these are waters receiving drainage from mining operations. Such systems have not been examined.

- (8) Available information indicates that acute toxicity to aquatic organisms resulting from environmental concentrations of NTA is unlikely. Similar studies on chronic toxicity suggest the same. Elucidation of the mode of toxicity would allow a better evaluation of the likelihood of chronic toxicity to organisms not specifically tested under various environmental conditions.



It is evident that not all aquatic organisms can be tested for their susceptibility to NTA. Similarly, all the environmental conditions can not be tested. Thus it is important to develop principles to assist in prediction of toxicity, chronic or acute, but especially the former. Without such information, we are forced to rely on empirical observations.

- (9) NTA is not expected to increase eutrophication by providing nutrients from its decomposition. Furthermore, most experiments with simple systems indicate that it does not stimulate algal growth. However, long term studies using whole ecosystems have not been performed to the extent of satisfying the Task Force that NTA will not cause significant changes in aquatic community structure. Such experiments are necessary.

One of the least satisfying aspects of the testing of detergent builders has been that on the aquatic community. Too often studies have been done using single species of algae, and when community studies were done they were either unrealistic, too short, insufficiently studied, or omitted important aspects of the community. Studies of the results to date do not raise any serious concerns, but the final answer is not yet in.

NTA is low pH. A second concern is that the detergent builders used in NTA are based on a method developed by Warren and Maler (1972) and modified by Lee et al. (1973). NTA is compared to the detergent builders used in the study by Lee et al. (1973). Warren and Maler indicate a significant difference in the results of the two studies. The results of the study by Lee et al. (1973) are as follows:

An interlaboratory comparison of these two methods was conducted by Lee et al. (1973) and the results are reported by Lee et al. (1973). The results of the study by Lee et al. (1973) are as follows: At levels above 10 ppm, statistical analysis indicated that the results of the two studies are significantly higher. The results of the study by Lee et al. (1973) are as follows:

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## THE CHEMISTRY OF NTA IN WATER

### ANALYTICAL CHEMISTRY OF NTA

At least two different methods have been used for the determination of NTA in detergents, wastewaters, and natural waters. One method, used extensively at the Canada Centre for Inland Waters (CCIW), is based on the formation of a bismuth-NTA complex at low pH and analysis by differential cathode-ray polarography after the method of Afghan and Goulden (1971). The detection limit is 10  $\mu\text{g}/\text{l}$ . Iron(III) interferes by forming complexes with NTA at low pH. A second procedure, employed in the Procter and Gamble Company, is based on a method developed by Warren and Malec (1972) and modified by Aue *et al.* (1972). NTA is converted to the tributyl ester for determination by gas chromatography. Warren and Malec indicate a detection limit of 25  $\mu\text{g}/\text{l}$ ; modifications by Aue *et al.* have lowered this to about 1  $\mu\text{g}/\text{l}$ .

An interlaboratory comparison of these two methods was performed by CCIW and Procter and Gamble. The results as reported by Matheson (1977) are not in good agreement especially at low concentrations on the order of 10 ppb and less. At levels above 10 ppb various statistical analyses confirmed that the Procter and Gamble values are significantly higher. The two could be predicted from each other by regression analysis, however. Possible causes include:

- (1) accidental contamination of samples;
- (2) the methods do not respond to the same species or have different interferences;
- (3) changes during storage.

Perry (1977) of Procter and Gamble has also performed a useful statistical analysis of the results from both laboratories. Based on a t-test of samples in which both laboratories reported values  $> 10 \mu\text{g}/\text{l}$ , the Procter and Gamble data were higher on the average than the CCIW values. The association between the two values was statistically significant; the correlation coefficient was about 0.7. However, the differences between the measurements can be considerable. Using regression analysis, Perry notes, for example, that if Procter and Gamble were to measure 50  $\mu\text{g}/\text{l}$  in a sample, the predicted CCIW value would be 39  $\mu\text{g}/\text{l}$ . The 95% confidence interval for the true CCIW average would be 33 to 47  $\mu\text{g}/\text{l}$ , and the 95% interval for an individual measurement by CCIW would be 10 to 148  $\mu\text{g}/\text{l}$ .



## CHEMICAL EQUILIBRIA

### SOLUTION CHEMISTRY

The aqueous chemistry of NTA is dominated by its reactions with metals including the proton. Calculating the equilibrium state of a water containing metals, NTA, and other ligands can be complex, so that numerical methods are frequently used. Important examples include Childs (1971), Lerman and Childs (1973), and Winters (1977a).

Childs (1971) performed calculations using a model lake water with major ion chemistry (calcium, magnesium, inorganic carbon, sulfate) similar to the Great Lakes. At pH 8 and for NTA concentrations less than  $10^{-6}$  moles/l, most of the NTA is calculated to be complexed with  $\text{Cu(II)}$ . Some complexation of Pb also occurs. At concentrations of NTA from  $2 \times 10^{-5}$  to  $2 \times 10^{-4}$  moles/l, the NTA is complexed primarily with calcium because there is insufficient copper present to react with it. Although not stated by the author, all of the copper present in the water ( $2 \times 10^{-6}$  moles/l) is presumably complexed with NTA at these higher NTA concentrations. Calculations for pH 6 indicate that Cu is again complexed with NTA at lower NTA concentration, but "free" NTA (i.e.  $\text{HNTA}^{-2}$ , NTA complexed with one proton) predominates at higher concentrations ( $2 \times 10^{-4}$  moles/l). Similar results are reported by Winters (1977a) for a water with similar major ion chemistry. In addition to Cu and Pb, some complexation of Ni by NTA at low concentration ( $<10^{-7}$  moles/l) is also indicated. Of additional interest are the concentrations of free aquometal ions in these solutions. For example, Winters (1977b) calculates that  $[\text{Cu}^{2+}]$  decreases from  $10^{-8.3}$  to  $10^{-12.3}$  moles/l as total NTA increases from  $10^{-8}$  to  $10^{-4}$  moles/l. This spectrum covers the range from the detection limit to concentrations in domestic wastewater.

Possible effects of complexation on degradation have been examined conceptually by Lerman and Childs (1973). A natural water will contain a mixture of NTA-metal complexes such as  $\text{HNTA}^{-2}$ ,  $\text{CaNTA}^{-}$ , and  $\text{CuNTA}^{-}$ . These different chemical species may degrade at different rates. Lerman and Childs consider the case when equilibrium is attained among the metal ions and NTA as degradation of NTA progresses, and also degradation of individual species without the reestablishment of equilibrium as decay occurs. Decay with reestablishment of equilibrium is predicted to occur faster. However, it is possible that degradation of NTA at low concentrations (say  $10^{-7}$  moles/l total NTA) may still be slow if some metal-NTA complexes (such as  $\text{CuNTA}^{-}$ ) are slowly degradable, because these predominate and the concentrations of other, more biodegradable NTA complexes are very low.

These results suggest cautious interpretation of results in at least three areas. First, estimates of metal-NTA complexation cannot be made solely on the basis of the equilibrium between the metal and NTA. For example, the iron (III)-NTA complex has a very large thermodynamic formation constant, but does not exist in appreciable concentration in natural waters at pH 8 because hydroxide has an even stronger affinity for Fe(III) than NTA. Speciation depends on the interaction among all metals and ligands in the system. Second, some laboratory studies of NTA degradation of specific metal-NTA complexes may have erred in assuming that a metal-NTA complex added



to a growth medium will remain in its original form. Rather, the metal and the NTA will reestablish equilibrium with the ligands and metals in the growth medium. These unknown species formed in the growth medium are those that are actually studied. Finally, NTA concentrations observed at the level of  $10^{-7}$  moles/l in Canadian waters are very likely complexes with such metals as Cu, Ni, and Pb, and may be very slowly degradable under those circumstances.

#### EFFECTS OF SOLIDS

Solids, whether suspended in the water column or settled in sediments, serve as possible sources of metals to solution and as possible sinks for NTA and NTA-metal complexes by adsorption. For example, Chau and Shiomi (1972) added NTA to polyethylene tubes suspended in Hamilton Harbor and observed that Zn and Fe were released from the underlying sediments. As the NTA was degraded, the metal concentrations also decreased. Laboratory experiments using lake water and sediments showed increased concentrations of Cu, Fe, Ni, and Zn. NTA dosages were in the order of 50  $\mu$ moles/l; increases in metal concentration were about 1  $\mu$ mole/l or less. It is probable that Cu, Ni, and Pb were mobilized as soluble NTA-metal complexes. For iron, this is less likely unless the iron was present as Fe(II). Alternatively, the NTA may have peptized colloidal ferric oxides. In any event, the effect was small and reversible as degradation of NTA occurred in these oxidized environments.

Mobilization under reduced environments can be different. Dunlap *et al.* (1971) investigated the effects of passing NTA through different soils under oxic and anoxic conditions. Rapid and complete degradation occurred in unsaturated or oxic soils. Under saturated (anoxic) conditions, degradation of NTA was negligible and significant mobilization of metals occurred under some conditions. When the soils contained metals as solid sulfides, mobilization was not great even under anoxic conditions. Apparently sulfide was able to compete with NTA for the metals and maintained them in solid phases. However, when the soils contained metals from organic sludges, extensive mobilization occurred. Sulfide, then, is another important ligand to be considered in metal mobilization.

Studies of the adsorption of NTA on solids are difficult to evaluate, as in most cases the solids and the adsorbing NTA species have not been well defined. One exception is a study by Huang and Ho (1977). These investigators studied the adsorption of uncomplexed NTA on silica, calcium carbonate, alumina, and kaolinite. Adsorption occurred on all solids. Adsorption depended on pH and solid type, and generally did not exceed about 30%.

Dunlap *et al.* (1971) noted adsorption of NTA on loam and loam-clay soils. This adsorption led to enhanced degradation under oxic conditions. Neither the NTA species nor the chemical composition of the solids were reported.

Results of monitoring NTA removal in wastewater treatment plants in Canada (Brownridge, 1977) indicate substantial removal of NTA in primary treatment and thereby suggest adsorption of NTA on sewage particulates. Bouveng *et al.* (1968) report adsorption of NTA on activated sludge; the extent of adsorption was about 30%. Gledhill (1977) mixed primary and



activated sludges with  $^{14}\text{C}$ -labelled NTA and observed only very small amounts of adsorption. All of these results are difficult to interpret, as the NTA species, the solution composition, and the organic particulates were not characterized. It seems plausible, however, that adsorption of NTA can occur on organic particulates in some cases. The specific conditions (pH, metal composition, type of organic particulate, other soluble organics, etc.) are not known.

#### APPLICATIONS TO AQUATIC SYSTEMS

Measurements and models both indicate that the effects of NTA on aquatic systems will be influenced by other chemical constituents in these systems. Important factors include pH, major cations, trace cations, inorganic ligands, other organic ligands, redox conditions, and the presence of inorganic and organic particulates. The effects of NTA on fresh, moderately hard, somewhat alkaline, oxic waters containing low concentrations of humic and other organics have been documented in Canadian surface waters. Somewhat persistent low concentrations of NTA are indicated, possibly due to the formation of trace metal-NTA complexes that are slowly degradable. Extrapolation of these results to other conditions should be made with some caution, examples follow:

##### 1. Hard vs. Soft Water

Hardness does not affect NTA speciation at low NTA concentrations ( $10^{-6}$  moles/l). At high NTA concentrations hard waters will produce  $\text{CaNTA}^-$  and  $\text{MgNTA}^-$  complexes while soft waters, such as those in eastern U.S.A. will produce protonated NTA complexes such as  $\text{HNTA}^{2-}$ . It is probable that this does not exert major effects on the fate of NTA and trace metals in aquatic systems.

##### 2. Oxic vs Anoxic Waters

Anoxic conditions produce direct effects on several metals and ligands in water, and these changes can exert effects on NTA. For example, metals such as iron, manganese, and chromium are reduced. Iron and manganese become more soluble; chromium is less soluble. Effects of this type change metal concentrations in the water and hence should change NTA speciation. Sulfate is reduced to sulfide under anoxic conditions; sulfide in turn precipitates metals such as iron(II) and lead. Phosphate may increase as organic matter is decomposed, and then form complexes and precipitates with metals. NTA is not readily degraded under anoxic conditions, so that it may accumulate. No systematic study of such anoxic effects has been made; it is very likely that they will be significant.

##### 3. Soluble Organics

Other organics in water, both those introduced by man and those produced naturally, can form soluble complexes with metals and may effectively compete with NTA to form metal complexes. Colored soft waters containing high concentrations of humic substances may respond differently to NTA than those Canadian surface waters which are low in such materials. It



is possible that organics in sewage may prevent the formation of NTA-trace metal complexes; removal of the sewage organics in biological wastewater treatment plants might then allow such complexes to form between any NTA and trace metals that pass through the plant. No studies of such effects appear to have been made.

#### 4. Fresh vs Saline Waters

The speciation of many substances in estuarine and oceanic waters is impossible to measure directly, although in some cases it can be calculated. It is likely that NTA speciation is affected significantly, but no studies, either experimental or conceptual, have been made.

#### 5. Acidic Waters

Some lakes and rivers develop low pH (4 or less) from atmospheric input of acid rain, or input of acid mine drainage. At low pH the NTA molecule becomes protonated, and does not interact as strongly with cations. One exception is Fe(III). The stability constant for the Fe(III)-NTA complex is very large. Hydroxide prevents formation of this complex at neutral pH, but it can form at pH 4. Acidic waters will thus contain Fe(III)-NTA complexes, and no others.

#### 6. Turbid Waters

The Colorado and Mississippi Rivers and many rivers in the southeastern U.S.A. contain substantial concentrations of suspended particulates. The effects of such suspended matter on the fate of NTA, and the effects of NTA on the metals associated with these particulates, are not known. Present knowledge of adsorption and mobilization phenomena suggests that these are not important, but it is possible that they may be.

#### 7. High Toxic Metal Concentrations

Waters containing high concentrations of metals such as Cu, Ni, and Pb can form correspondingly large concentrations of the metal-NTA complexes if NTA is added. It is possible that these may persist in the environment. Actual speciation will depend on other factors, such as the other organics in the system. A study of such a system would be useful.

### PHOTOCHEMISTRY

The photochemical degradation of Fe(III)-NTA complexes has been studied by Trott *et al.* (1972). Extensive degradation occurred over the pH range 4 to 8. Efficiency declined with increasing pH. Degradation products included iminodiacetic acid (IDA), formaldehyde, and CO<sub>2</sub>. These authors consider that the photochemical reaction originates from a charge transfer excited state complex. Langford *et al.* (1973) reported the results of similar studies of Cu(II)-NTA complexes. Photo-decomposition of NTA was observed. Efficiency decreased with increasing pH over the range 2 to 12, and also decreased with increasing NTA concentration. The results provide additional support for the charge transfer reaction, and therefore suggest that complexes of NTA with Ca<sup>2+</sup> and Mg<sup>2+</sup> will not react with sunlight.



Stolzberg and Hume (1975) have also studied the photochemical degradation of Fe(III)-NTA solutions. Rapid degradation of Fe(III)-NTA complexes occurred; the half life was about 1.5 hours at pH 5.2 to 5.8. The principal product was IDA. Very slow degradation of Fe(III)-IDA was observed, presumably because of the much lower stability constant of the Fe(III)-IDA complex. No photodegradation of Pb(II) and Cd(II) NTA complexes was noted. A marginally significant reduction was observed for Cr(III), and a 70 percent reduction of a 10 ppm NTA solution was observed in a week with a solution containing a 40-fold excess of Cu(II).

These results suggest that the photochemical degradation of Fe(III)-NTA complexes to IDA may be significant in acidic waters. A slow photochemical degradation of Cu(II)-NTA complexes in neutral waters is also plausible. The IDA that is produced is probably biologically degradable, at least in neutral waters. The fate of IDA produced in acidic waters should be considered.

#### CHLORINATION OF NTA

Warren (1976) has studied the effects of ammonia, metal ions, pH, and sunlight on the reactions of NTA, IDA, and glycine with chlorine. In the absence of complexing metal ions and ammonia, chlorination of NTA was rapid, producing IDA, N-chloro IDA, and glycine. These products did not accumulate, since their reactions with chlorine were even more rapid than NTA. Ammonia impaired the reaction by forming chloramines that did not react with NTA. Divalent metal ions retarded the reaction by forming metal-NTA complexes, coordinating the nitrogen atom of NTA that apparently is the initial reaction site for free chlorine. These results suggest that NTA will not be oxidized by chlorine in wastewater effluents because of the presence of ammonia, or in potable water supplies because of the existence of metal-NTA complexes.

#### REFERENCES

- Afghan, B. K., and P. D. Goulden, 1971. Determination of Trace Quantities of Nitrilotriacetic Acid by Differential Cathode-Ray Polarography, *Env. Sci. and Technol.*, 5: 601-606.
- Aue, A., C. R. Hastings, K. O. Gerhardt, J. O. Pierce, H. H. Hill, and R. F. Moseman, 1972. The Determination of Part-Per-Billion Levels of Citric and Nitrilotriacetic Acids in Tap Water and Sewage Effluent. *J. of Chromatog* 72: 259-267.
- Bouveng, H. O., G. Davisson, and E. Steinberg, 1968. NTA in Sewage Treatment. *Vatten*, 22: 348-359.
- Brownridge, F. A., 1977. Concentrations of NTA in Canadian Waters. In: Presentations Prepared by Monsanto and Procter and Gamble Companies for the IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders.
- Chau, Y. K., and M. T. Shiomi, 1972. Complexing Properties of Nitrilotriacetic Acid in the Lake Environment; Water, Air, and Soil Pollution. 1: 149-164.



- Childs, C. W., 1971, Chemical Equilibrium Models for Lake Water Which Contains Nitrilotriacetate and for "Normal" Lake Water. Proc. 14th Conf. on Great Lakes Research; 198-210.
- Dunlap, J., R. L. Cosby, J. F. McNabb, B. E. Bledsoe, and M. R. Scalf, 1971. Investigation Concerning Probable Impact of Nitrilotriacetic Acid on Ground Waters. Environmental Protection Agency, Water Pollution Research Series # 16060 GHR.
- Gledhill, W. E. 1977. NTA Adsorption to Primary Sludge and Activated Sludge. Monsanto Company Internal Report.
- Huang, C. P., and A. Ho, 1977. Adsorption Characteristics of Nitrilotriacetate (NTA) at Solid Solution Interfaces, submitted to Journal of Colloid and Interface Science.
- Langford, H., M. Wingham, and V. S. Sastri, 1973. Ligand Photooxidation in Copper(II) Complexes of Nitrilotriacetic Acid: Implications for Natural Waters. Env. Sci. and Technol., 7: 820-822.
- Lerman, A., and C. W. Childs, 1973. Metal-Organic Complexes in Natural Waters: Control of Distribution by Thermodynamic, Kinetic, and Physical Factors. In: "Trace Metals and Metal-Organic Interactions in Natural Waters", edited by P. C. Singer, Ann Arbor Science, 201-235.
- Matheson, D. W., 1977. Nitrilotriacetic Acid (NTA) in the Canadian Environment. Scientific Series No. 74, Environment Canada.
- Perry, L., 1977, Communication to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders.
- Stolzberg, R. J., and D. M. Hume, 1975. Rapid Formation of Iminodiacetate from Photochemical Degradation of Fe(III) Nitrilotriacetate Solution. Env. Sci. and Technol., 9: 654-656.
- Trott, T., R. W. Henwood, and C. H. Langford, 1972. Sunlight Photochemistry of Ferric Nitrilotriacetate Complexes. Env. Sci. and Technol., 6: 367-368.
- Warren, C. B., 1976. Chlorine Oxidation of Nitrilotriacetate (NTA) and Related Imino and Amino Acids, Monsanto Internal Report.
- Warren, C. B. and E. J. Malec, 1972. Biodegradation of Nitrilotriacetic Acid and Related Imino and Amino Acids in River Water. Sci., 176: 277-279.
- Winters, R. E., 1977a. Chemistry of NTA. In: Presentation Prepared by Monsanto and Procter and Gamble Companies for the IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders.
- Winters, R. E., 1977b. Communication to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders.







# MICROBIOLOGY AND BIOCHEMISTRY OF NTA DEGRADATION

## MICROBIOLOGY OF NTA-DEGRADING ORGANISMS

The principal biological agents responsible for the complete degradation of the majority of organic compounds in the environment, both of natural occurrence and of synthetic origin, are recognized as bacteria and fungi. It is within these groups of microorganisms that the requisite genetic potential for the elaboration of degradative enzymes resides, since such organisms frequently accomplish complete degradation as a means of satisfying their requirements for carbon, nitrogen and energy.

Aerobic degradation of organic compounds by microorganisms can thereby result in complete oxidation to water, carbon dioxide, inorganic forms of nitrogen, and conversion of the remainder to microbial biomass. That this is the case, for some if not all NTA-degrading microorganisms present in oxygen rich situations where NTA is known to occur, can be documented by reference to numerous literature reports of the isolation of pure cultures of aerobic bacteria growing at the expense of NTA (Forsberg & Lindquist, 1967a, 1967b; Focht and Joseph, 1971; Wong et al., 1972; Cripps & Noble, 1973; Enfors & Molin, 1973a; Parks & Sturus, 1973; Tiedje et al., 1973).

Since all the organisms thus far described have been isolated by procedures which deliberately select for those which utilize NTA for growth, it is not possible to state at this time whether other organisms may be found which possess the capacity merely to modify or alter the structure of the NTA molecule without deriving any energetic or nutritive benefit from the process. This would appear likely, however. Pickaver (1976) has reported the isolation of Pseudomonas, and Bacillus and yeast strains able to use NTA and iminodiacetic acid as their sole carbon source but not as their sole carbon and nitrogen source, suggesting that they accumulate a nitrogenous product (glycine?). Of the various aerobic microorganisms isolated and characterized from sources such as soil, mud, natural waters, and sewage, all are bacteria or yeasts; no examples of fungi, algae or protozoa utilizing or modifying NTA have been reported. Notably where these bacterial isolates have been further classified, a high proportion are found to fall in the genus Pseudomonas, a group of Gram negative, aerobic, motile, rods, long recognized as organisms versatile in the degradation of organic compounds (Stanier et al., 1966). Because of their rapid division rates with a wide variety of organic compounds as sole carbon source, these bacteria are frequently the principal organisms found by traditional enrichment culture methods. It should be stressed here that alternative isolation methods may yield representatives of other bacterial genera.



The nutritional requirements for members of the genus Pseudomonas are simple; they do not require cofactors, amino acids, or vitamins for growth, and grow readily in simple, chemically defined, mineral salts media. Evidently bacteria capable of NTA degradation are present in soils, fresh water and sewage. Experience with NTA as a chemical useful for maintaining trace metal solubility in bacteriological growth media indicates that the number of stock bacterial cultures able to use NTA is low. Similarly in populations of bacteria taken from sewage the occurrence of NTA-utilizers is infrequent no doubt contributing to the variations reported for the ease with which NTA-degrading microflora are established in activated sludge and other treatment processes.

The currently accepted pathway for the degradation of NTA by aerobic bacteria is that based on biochemical studies with pure cultures of different strains of Pseudomonas (Focht & Joseph, 1971; Tiedje et al., 1973; Cripps & Noble, 1973); Firestone and Tiedje, 1978). It should be emphasized that a real possibility exists that alternative pathways for NTA degradation may operate in other bacterial genera and that only by a systematic microbiological and biochemical study would such alternatives be revealed. Numerous examples of alternative catabolic pathways used by different bacterial genera for a given growth substrate can be found in the literature of microbial metabolism. It is possible, therefore, that other NTA metabolites might be recognized from such studies.

The isolation of pure bacterial cultures capable of anaerobic growth with NTA is documented in only one report (Enfors & Molin, 1973a). The organisms isolated were capable both of aerobic growth with NTA as sole carbon and nitrogen source and of anaerobic growth only with nitrate provided as an alternative to oxygen as electron acceptor. The bacteria were not characterized beyond their description as gram negative, motile rods.

## BIOCHEMISTRY OF NTA-DEGRADATION

### UPTAKE OF NTA BY MICROORGANISMS

Studies with organisms able to grow with NTA have provided evidence that its permeation into bacterial cells is energy dependent since respiratory inhibitors (cyanide and azide) drastically reduce the rate and level of its uptake (Wong et al., 1973). If active transport is involved, bacteria may be able to concentrate NTA from an environment where its concentration is low. Measurements of the rates of oxidation of NTA<sup>0</sup> by a Pseudomonas as a function of NTA concentration show that at 30°C half-maximal rates ( $K_M$  for intact cells) are found at 18  $\mu\text{M}$  NTA (3.4 ppm) where the maximum rate of oxidation is 2.93  $\mu\text{mole O}_2$  per hr per mg dry wt. (66  $\mu\text{liters O}_2/\text{hr/mg cell dry wt.}$ ) (Firestone & Tiedje, 1975). This rate is notably larger than that found with a Pseudomonas mutant selected for its ability to grow rapidly with high concentrations (2.5%) of NTA (34  $\mu\text{liters O}_2/\text{hr/mg dry wt. at } 25^\circ\text{C}$ ) (Liu et al., 1973). These authors have reported a  $K_M$  for NTA uptake by intake cells of 82  $\mu\text{g/l}$  (0.43  $\mu\text{M}$  or 82 ppb) a value significantly different from the value reported by Firestone & Tiedje (1975) and revealing a remarkable capacity of these cells to transport NTA at very low concentrations (For E. coli active transport of lactose is half maximal at an external concentration of 70  $\mu\text{M}$ . Hence NTA is concentrated by this Pseudomonas about 200x more efficiently).

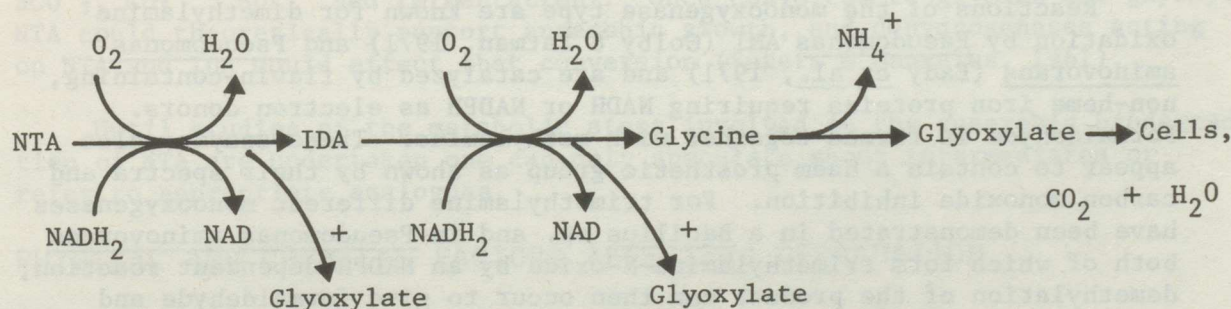


For compounds such as glycine, glycolate and acetate Wright (1975) reports  $K_M$  values for cells of freshwater bacteria in the range established by Wong et al. (1973) for NTA.

#### AEROBIC DEGRADATION; PATHWAYS AND ENZYMES

Based on preliminary studies with intact cells of NTA-grown *Pseudomonas* strains, and a variety of chemically feasible intermediates, the research groups of Focht (Focht & Joseph, 1971) and Tiedje (Tiedje et al., 1973) both inferred that the degradative pathway involved iminodiacetic acid as the first intermediate, and that decarboxylation of NTA to such compounds as N-methyl iminodiacetic acid and sarcosine did not occur. Cells grown in the absence of NTA oxidized this compound only after a lag period suggesting that induction of the necessary enzymes was necessary (Tiedje et al., 1973; Cripps & Noble, 1973).

Confirmation that the reaction pathway involved two sequential cleavages of the C-N bonds of NTA was obtained from studies with enzymes present in cell extracts of NTA-grown cells (Cripps & Noble, 1973; Tiedje et al., 1973; Firestone and Tiedje, 1978; glyoxylate was identified as the cleavage product in the first reaction leading to formation of iminodiacetic acid (IDA). An analogous reaction follows which yields a second molecule of glyoxylate from iminodiacetic acid with glycine as the other product. Both glycine and glyoxylate are naturally-occurring compounds and their utilization by bacterial cells is well established (Callely et al., 1961).



SCHEME 1.

Earlier demonstration of an oxygen requirement by enzymes in cell extracts acting on NTA (Cripps & Noble, 1973) has been followed up by studies of purified enzymes in Tiedje's laboratory (Tiedje, 1977; Firestone et al., 1978).

The enzyme which accomplishes NTA oxidation has been partially purified and shown to be a manganese ion-dependent flavoprotein catalyzing an NADH-dependent mono-oxygenation of NTA (see scheme 1). It behaves as a typical flavoprotein monooxygenase and may be thought of as catalyzing the oxidation of a methylene group in NTA to a hydroxymethylene giving an unstable product which then yields glyoxylate and IDA. No evidence for the participation of NTA-N-oxide in this reaction has been obtained. The NTA monooxygenase also acts on IDA and again requires  $Mn^{++}$  (or  $Mg^{++}$  for Cripps and Noble's organism).



for this reaction which yields glycine plus glyoxylate. Its requirements for molecular oxygen as a substrate in both the first and second reactions of NTA degradation demonstrate the importance of adequately aerobic conditions for NTA breakdown by these organisms.

Studies with the purified enzyme have yielded information about its relative affinities for NTA ( $K_M = 32 \mu M$ ), IDA ( $K_M = 500-800 \mu M$ ) and Mn ( $K_M = 30 \mu M$ ) suggesting that the mechanism of concentration of NTA by intact cells ( $K_M = 18 \mu M$ ) is one of active transport and not rate limiting.

There is no evidence to show that decarboxylation of NTA or its metabolites can occur to give such products as N-methyl iminodiacetic acid, dimethylglycine or N-methylglycine (sarcosine); this is significant in view of the fact that N-nitroso-sarcosine is recognized as a carcinogen (Druckrey *et al.*, 1963).

#### DEGRADATION OF STRUCTURALLY RELATED COMPOUNDS

Consideration of the biochemical mechanisms utilized by bacteria for the degradation of secondary and tertiary amines other than IDA and NTA reveals that both monooxygenase and dehydrogenase reactions are known (Large, 1971). Such examples can provide useful analogies for chemically and biologically feasible alternatives for NTA degradation. Clearly such alternatives must exist if biodegradation of NTA is to proceed under anaerobic conditions where oxygen dependent reactions of the type elucidated by Tiedje cannot apply.

Reactions of the monooxygenase type are known for dimethylamine oxidation by *Pseudomonas* AML (Colby & Zatman, 1971) and *Pseudomonas aminovorans* (Eady *et al.*, 1971) and are catalyzed by flavin-containing, non-heme iron proteins requiring NADH or NADPH as electron donors. Formaldehyde is formed together with methylamine. These enzymes also appear to contain a haem prosthetic group as shown by their spectra and carbon monoxide inhibition. For trimethylamine different monooxygenases have been demonstrated in a *Bacillus* sp. and in *Pseudomonas aminovorans* both of which form trimethylamine-N-oxide by an NADPH-dependent reaction; demethylation of the product can then occur to give formaldehyde and dimethylamine (Large *et al.*, 1972; Myers & Zatman, 1971). The N-oxygenation reaction has been most carefully characterized in a hog liver microsomal system by studies with  $^{18}O_2$  (Baker and Chaykin, 1962).

The above examples illustrate close analogies with the steps of aerobic degradation of NTA. There are, however, examples of secondary and tertiary amine metabolism where oxygen does not play a role as a substrate. Thus in a non-motile gram-negative bacterium, trimethylamine is demethylated by a tertiary amine dehydrogenase using artificial electron acceptors (Colby & Zatman, 1971). Similar dehydrogenases have been described for the secondary amines, spermidine (Tabor & Kellogg, 1970) and sarcosine (Frisell, 1971; Hall, Simpson & Crosbie, 1971) and the tertiary amine, N, N-dimethylglycine (Hall, Simpson & Crosbie, 1971). In view of the existence of such dehydrogenases which effect C-N bond cleavage by generating hydrolyzable imines and ultimately yielding



aldehydes and the correspondingly less substituted amines, one can propose chemically feasible routes for the anaerobic degradation of NTA. In line with current views of the ways in which bacteria evolve new catabolic enzymes and pathways by recruiting old enzymes, it is not unreasonable to speculate that the currently accepted pathway of aerobic degradation of NTA may have evolved from enzymes which had as their 'normal substrates' compounds such as dimethylglycine or trimethylamine.

## ANAEROBIC DEGRADATION; A SPECULATION ON PATHWAYS INVOLVED

As mentioned earlier very little is known of the anaerobic degradation of NTA by pure cultures of bacteria (Enfors & Molin, 1973a, 1973b). In these studies facultatively anaerobic bacteria were shown to be capable of growing with NTA under anaerobic conditions provided nitrate was present as an electron acceptor. From the foregoing considerations of reactions by which tertiary amines can be dissimilated without requiring molecular oxygen, it would appear that such reactions may be responsible for NTA degradation anaerobically. Unfortunately the above authors have presented no evidence to show what biochemical reactions may be involved in NTA degradation, either aerobically or anaerobically by their bacterial strains.

Since a number of literature reports document the anaerobic degradation of NTA in soils (Tabatabai & Bremner, 1975); in sewage systems (Claesson, 1971, Moore & Barth, 1976); and septic tanks (Klein, 1974, Thompson & Jones, 1971), environments where the concentration of nitrate would not appear to be normally sufficient for nitrate-dependent anaerobic respiration, there are apparently other anaerobic mechanisms operative (anaerobic respiration with  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$  and fermentation). Once converted to glycine and glyoxylate NTA could theoretically support anaerobic growth, and dehydrogenases acting on NTA and IDA could effect that conversion (Sagers & Gunsalus, 1961).

Until studies of the metabolic steps involved in the anaerobic biodegradation of NTA are undertaken one can only speculate about intermediates or refer to appropriate analogues.

## PHYSICAL AND CHEMICAL FACTORS AFFECTING DEGRADATION

### HYDROGEN ION CONCENTRATION AND pH

Pure cultures of NTA-utilizing bacteria are capable of growth over pH ranges normally encountered in the environment. The *Pseudomonas* strain isolated by Focht and Joseph (1971) will grow with NTA in the pH range 6.5-8.5; that of Wong *et al.* (1972) tolerates a pH range of 5 to 9 for NTA growth with optimal growth at pH 7.0.

### SALINITY

The degradation of NTA by marine organisms in marine environments has been reported (Erickson *et al.*, 1970), yet under estuarine conditions NTA degradation is apparently insignificant (Gledhill & McDonald, 1976; Bourquin & Przybyszewski, 1977). Since freshwater bacteria capable of NTA degradation were apparently much less effective in degrading this compound in saline



environments it would appear that an effect of salinity on NTA-degrading enzymes was responsible. Currently studies by Tiedje's group (Tiedje, personal communication) suggest that mono-oxygenase acting on NTA is subject to inhibition at high ionic strength, Firestone et al., 1978. Anion inhibition of NADP dependent mono-oxygenases has been reported by others (Kamin, 1971; Steenis et al., 1973).

#### OXYGEN CONCENTRATION

With the demonstration that the degradation of NTA involves two oxygen-consuming reactions (at least in the organisms studied by Cripps & Noble; 1973, and that by Firestone, 1975) an absolute dependence on oxygen is to be expected for NTA metabolism in these strains. No determinations of  $K_M$  values for oxygen with intact cells or enzyme preparations have been reported. Experiments with soil microflora show that oxygen does not limit degradation when it is perfused at concentrations as low as 1% (Tiedje & Mason, 1974). At 0.1%  $O_2$  degradation rates are slowed suggesting that the microflora acclimated in the first 8-10 days under air uses an oxygen dependent route of degradation.

#### CONCENTRATION OF NTA

Bacteria able to utilize concentrations of NTA as high as 2.5% have been described (Wong et al., 1972). More usually pure cultures have been isolated which grow with NTA at 0.2% (Focht & Joseph, 1971), or 1.0% (Tiedje et al., 1973). While such high concentrations are unlikely to be encountered by more widespread use of NTA as a detergent builder, clearly high NTA concentrations present no problem to pure cultures selected for that ability. The question of how effectively low concentrations of NTA (0-1 mg/liter) may be removed has not been addressed in pure culture studies other than those reported by Wong et al., 1973. Since the organism chosen for their work was selected for utilization of high concentrations of NTA it may not be typical of strains capable of growth with low concentrations. Additional studies of the utilization of NTA, and indeed other organic compounds, at low nutrient concentrations are required to determine whether organisms with a greater potential to concentrate nutrients can remove the low concentrations of NTA in water leaving treatment facilities. In the absence of such organisms the rates of degradation of NTA will be seen to decrease linearly with decreasing concentration below comparatively high thresholds. Such is the case apparently in laboratory investigations of NTA degradation by aquatic communities (Patrick et al., 1976). At 0.02 mg/l NTA its degradation was 250 times slower than at 20 mg/l. Without a more detailed examination of the kinetics of this process the threshold concentration of NTA remains indeterminate. One value of  $K_M$  for bacterial oxidation of NTA by cells is given as 18  $\mu M$  (4.3 mg/l) (Firestone & Tiedje, 1975).

#### DEGRADATION OF NTA-CHELATES

NTA is considered a useful alternative to phosphate-containing detergent builders because it is a good chelating agent for divalent metals, yet it is precisely this property which prompts questions about its ability to mobilize metals from sediments and soils with environmental and health consequences. Concerns have also been expressed about the effect of metals on the biodegradability of NTA.



Warren (1974) has reviewed the literature on microbial degradation of metal chelates of NTA pointing out the importance of understanding more fully the nature of NTA uptake by cells and the participation of different metal complexes. Since the enzymes of NTA oxidation in a *Pseudomonas* are located in the soluble fraction within the cell (Cripps & Noble, 1973; Tiedje *et al.*, 1973) it follows that for degradation of various NTA chelates they must either be transported and attacked at similar rates or exchange processes must occur outside the cell membrane such that NTA always reaches the internal enzymes in the same form. The latter process appears to apply in the *Pseudomonas* studied by Tiedje's group. At concentrations of chelates low enough ( $2 \times 10^{-5}$  M) to prevent toxicity, the NTA complexes of Ca, Mn, Mg, Cu, Zn, Cd, and Fe were degraded at very similar rates. The Ni complex was not degraded at this concentration. At higher concentrations ( $10^{-3}$  M) the Cu, Cd and Zn complexes were metabolized more slowly than the trisodium salt of NTA and the addition of soil increased these rates by differing degrees. Evidence that NTA does not facilitate uptake of metals by cells was also obtained, supporting the concept of a NTA-binding protein at the membrane surface which destabilizes the metal-NTA complex.

The effect of soil on stimulating the metabolism of certain metal chelates was attributed to its binding of toxic cations. Previous studies (Tiedje & Mason, 1974) had shown that a variety of metal-NTA complexes were metabolized by soils although the Cd- and Hg-chelates required longer lag periods and were degraded more slowly than others.

Generally the complexes of NTA with Cu, Zn, Ni, and Cd are those which show lowered or even negligible rates of oxidation under different conditions. This may be attributed to the ability of Cu, Ni, Zn and Cd ions to form strong tetradentate complexes with NTA at pH 7.0; log K varies from 7-11 as compared to the weaker tridentate complexes (log K from 2-6) formed by Fe, Ca and Mn ions whose complexes are more readily degraded. On the other hand, the effect may be as for mercury where the slow degradation of the  $Hg_2^{++}$  complex is apparently due not to its stability but to the toxic effects of  $Hg^{++}$  on cell metabolic processes.

The addition of soil, sediments, activated sludge, or Ca and Mg ions can improve the bacterial degradation of the less readily attacked NTA chelates by metal ion transfer or by ligand exchange or perhaps even by inactivation of the toxic ions by adsorption. The first process allows for the chelation of some fraction of NTA with another ion to give a complex which is more readily degradable. This was realized by Huber & Popp (1972) who added Ca ions to sewage and thereby facilitated the degradation of NTA originally present as its Cd chelate. Ligand exchange involves a process in which metal ions are redistributed in the system between NTA and other ligands. A variety of organic and inorganic ligands in soils, sediments and activated sludge can compete with NTA for various cations and thereby render the persistent complexes more degradable. Degradation of NTA-complexes will therefore be more complete in various environments where ligand exchange and metal ion exchange can occur.

## PRODUCTS AND RESIDUES FROM NTA

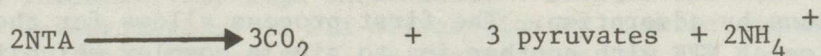
Apart from NTA, iminodiacetic acid is the only other compound found in its degradation by pure bacterial cultures, which is unknown as a natural



product. It has been identified as the first nitrogen-containing metabolite of NTA formed by aerobes (see A ii) and is a predicted intermediate if a dehydrogenase type attack on NTA occurs anaerobically (see C). Concern about the possibility that this secondary amine may form, under certain environmental conditions a N-nitroso-derivative with potential carcinogenic properties, was first expressed by Epstein (1972). Numerous attempts to demonstrate its accumulation, transient or otherwise, from NTA in pure cultures and natural environments have been unsuccessful, probably because IDA is metabolized at least as rapidly as NTA within cells (Focht & Joseph, 1971; Tiedje *et al.*, 1973; Cripps & Noble, 1973; Warren & Malec, 1972; Tiedje & Mason, 1974; Wong *et al.*, 1973).

Pickaver (1974, 1976) has reported the persistent accumulation of N-nitroso-iminodiacetic acid (~50 µg/ml) in soil percolators perfused with NTA and nitrate, or from IDA plus nitrite at levels of 100-200 mg/l of each. Pure cultures of five different organisms were isolated with the ability to form N-nitroso-IDA from nitrite and IDA whereas appropriately paired bacterial cultures were necessary before N-nitroso-IDA formation occurred from nitrate plus NTA. The ability to form N-nitroso-IDA was present in the cell extracts of only one of the partners and a second strain was apparently required to furnish some low molecular weight compound, possibly NO<sub>2</sub><sup>-</sup> or IDA, before synthesis took place. While Gledhill and McDonald (1971) also observed N-nitroso-IDA formation from nitrite and IDA in similar percolators, the nitrosated product was subsequently biodegraded. No significant formation of the nitroso product from NTA and nitrate was observed. Apparently the distribution of microorganisms able to convert NTA and nitrate to N-nitroso-IDA, and of those able to degrade this compound is not uniform. Nonetheless it is evident that although N-nitroso-IDA can be formed by certain microflora other bacterial populations exist to effect its biodegradation.

In pure cultures of NTA-utilizers the ultimate products of its degradation are carbon dioxide, water, ammonia and cell constituents (Focht & Joseph, 1971; Tiedje *et al.*, 1973). The nitrogen to carbon ratio of NTA (1:6) would suggest that all of the nitrogen would be assimilated into cell constituents but the fact that much of the carbon is already at a high oxidation level means that a considerable fraction is lost as carbon dioxide, a significant product of glyoxylate metabolism (see Scheme 1, IIIB) (Callely *et al.*, 1961). Accordingly an equation to show CO<sub>2</sub> formation based on the



established pathway reveals that ~1 N per pyruvate (N:C = 1:4.5) is available even before extensive cellular oxidation has taken place. Accumulation of N as NH<sub>4</sub><sup>+</sup> is, therefore, not surprising.

Reports of the conversion of NTA nitrogen to small quantities of nitrite in pure cultures (Focht & Joseph, 1971) and to nitrate in aerobic soils (Tabatabai & Bremner, 1975), and aerobic cultures of sewage organisms (Thompson & Duthie, 1968), are undoubtedly attributable to the action of aerobic heterotrophic and autotrophic nitrifying organisms acting on ammonia formed as the immediate product of NTA degradation. When soils are maintained under anaerobic conditions, ammonia rather than nitrate accumulates (Tabatabai & Bremner,



1975). As for levels of NTA remaining in soil and water beyond the point where NTA removal by vigorous microbial activity has occurred, monitoring studies (Matheson, 1977) show that concentrations from 0-1 mg/liter are found in waters below sewage treatment plants with most samples in the range 0-100 µg/liter. In drinking waters values from 0-60 µg/liter have been observed with most samples showing a range of 0-25 µg/liter. While these values are all below the  $K_M$  for NTA oxidation at 30°C (3.4 mg/liter) shown for *Pseudomonas* cells (Firestone & Tiedje, 1975) a second strain of *Pseudomonas*, obtained by mutagenesis, shows a  $K_M$  for NTA uptake of 82 µg/liter at 25°C (Wong *et al.*, 1973).

Thus the efficiency of NTA uptake mechanisms can vary from strain to strain and the potential for evolving bacterial strains with improved uptake appears to be a very real one. Concern has been expressed, however, (Dale Toetz, personal communication) that in natural environments having a low density of NTA-degrading organisms NTA will persist at low concentrations because of low degradation rates. Similar arguments apply to all organic materials whether natural or synthetic, hence the importance of such low level persistence must be carefully evaluated in the light of established effects on human health and aquatic environments.

## REFERENCES

- Baker, J. R. & S. Chaykin 1962. The Biosynthesis of Trimethylamine-N-Oxide. *J. Biol. Chem.* 237: 1309-1313.
- Bourquin, A. W. & V. A. Przybyszewski 1976. Distribution of Bacteria with Nitrilotriacetate-Degrading Potential in an Estuarine Environment. (Prepublication presented to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders. Chicago, Ill. 1977).
- Callely, A. G., P. W. Trudgill & S. Dagley 1961. Synthesis of Cell Constituents from Glycine by a *Pseudomonas*. *Biochem. J.* 81: 623-631.
- Claesson, A. 1971. Anaerobic Bacterial Degradation of Nitrilotriacetate NTA. *Vatten* 27: 410-411.
- Colby, J. & L. J. Zatman 1971. The Purification and Properties of a Bacterial Trimethylamine Dehydrogenase. *Biochem. J.* 124: 31P.
- Cripps, R. E. & A. S. Noble 1973. The Metabolism of Nitrilotriacetate by a *Pseudomonas*. *Biochem. J.* 136: 1059-1068.
- Druckrey, H., R. Preussman, R. Blum, S. Ivankovic, & J. Afkham 1963. Erzeugung von Karzinomen der Speiseröhre durch unsymmetrische Nitrosamine. *Naturwissenschaften* 50: 100-101.
- Eady, R. R., T. R. Jarman, & P. J. Large 1971. Microbial Oxidation of Amines. Partial Purification of a Mixed Function Secondary Amine Oxidase System from *Pseudomonas aminovorans* that contains an Enzymically Active Cytochrome P-420 Type Haemoprotein. *Biochem. J.* 125: 449-459.



- Enfors, S.O. & N. Molin 1973a. Biodegradation of Nitrilotriacetate (NTA) by Bacteria. I. Isolation of Bacteria Able to Grow Anaerobically with NTA as a Sole Carbon Source. *Water Res.* 7: 881-888.
- Enfors, S. O. & N. Molin 1973b. Biodegradation of Nitrilotriacetate (NTA) by Bacteria. II. Cultivation of an NTA-Degrading Bacterium in Anaerobic Medium. *Water Res.* 7: 889-893.
- Epstein, S. S. 1972. Toxicological and Environmental Implications of the Use of Nitrilotriacetic Acid as a Detergent Builder. *Internat. J. Environ. Studies* 2: 291-300.
- Erickson, S. J., T. E. Maloney, and J. H. Gentile 1970. Effect of Nitrilotriacetic Acid on the Growth and Metabolism of Estuarine Phytoplankton. *J. Water Poll. Cont. Fed.* 42: R329-R335.
- Firestone, M. K., S. D. Aust and J. M. Tiedje, 1978. A Nitrilotriacetic Acid Mono-oxygenase with Conditional NADH-Oxidase Activity. *Arch. Biochem. Biophys.* (In press).
- Firestone, M. K. and J. M. Tiedje 1975. Biodegradation of Metal Nitrilotriacetate Complexes by a Pseudomonas species. Mechanism of Reaction. *Appl. Microbiol.* 29: 758-764.
- Firestone, M. K. and J. M. Tiedje, 1978. Pathway of Degradation of Nitrilotriacetate by a Pseudomonas Species. *Appl. Environ. Microbiol.* 35: 955-961.
- Focht, D. D. & H. A. Joseph 1971. Bacterial Degradation of Nitrilotriacetic Acid (NTA). *Can. J. Microbiol.* 17: 1553-1556.
- Forsberg, C. & G. Lindquist 1967a. On Biological Degradation of NTA. *Life Sci.* 6: 1961-62.
- Forsberg, C. & G. Lindquist 1967b. Experimental Studies on Bacterial Degradation of NTA. *Vatten* 23: 265-277.
- Frisell, W. R. 1971. One Carbon Metabolism in Microorganisms. I. Oxidative Demethylation in a Sarcosine-Utilizing Bacterium. *Arch. Biochem. Biophys.* 142: 213-222.
- Gledhill, W. E. & P. D. McDonald 1971. Nitrosoiminodiacetate (NIDA) Formation in Soil Percolation Columns. Monsanto Internal Report, Aug., 13, 1975.
- Gledhill, W. E. & P. D. McDonald 1976. Microbial Degradation of Builder M and NTA in Laboratory Model Marine and Estuary Systems. Monsanto Internal Report - Interim Report #8448, Sept. 28, 1976.
- Hall, D. E., I. A. Simpson, & G. W. Crosbie 1971. Growth of Pseudomonas reptilovara on N-Methylglycines. *Biochem. J.* 124: 31P.
- Huber, W. & K. H. Popp 1972. Biological Degradation of Nitrilotriacetate Acid in the Presence of Cadmium Ions. *Fette Seifen. Anstr.* 74: 166-168.



Kamin, H., (1971) in Flavins and Flavoproteins (Kamin, H., Ed.) p. 472, University Park Press, Baltimore.

Klein, S. A. 1974. NTA Removal in Septic Tank and Oxidation Pond Systems. J. Water Pollut. Control Fed. 46: 78-88.

Large, P. J. 1971. The Oxidative Cleavage of Alkyl-nitrogen Bonds in Microorganisms. Xenobiotica 1: 457-467.

Large, P. J., C. A. Boulton, & M. J. C. Crabbe 1972. The Reduced Nicotinamide-Adenine Dinucleotide Phosphate- and Oxygen-Dependent N-Oxygenation of Trimethylamine by Pseudomonas aminovorans. Biochem. J. 128: 137P.

Liu, D., P. T. S. Wong & B. J. Dutka 1973. Studies of a rapid NTA-utilizing bacterial mutant. J. Water Pollut. Contr. Fed. 45: 1728-1735.

Matheson, D. H. 1977. Nitrilotriacetic Acid (NTA) in the Canadian Environment. A report presented before the IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders. 46 pp. Cincinnati, Ohio, 1977.

Moore, L. & E. F. Barth 1976. Degradation of NTA During Anaerobic Digestion. J. Water Poll. Contr. Fed. 48: 2406-2409.

Myers, P. A. & L. J. Zatman 1971. The Metabolism of Trimethylamine N-Oxide by Bacillus PM6. Biochem. J. 121: 10P.

Parks, S. M.J. & P. E. Sturus 1973. The Microbial Metabolism of Nitrilotriacetic Acid. J. Sci. Lab. Dension Univ. 54: 79-86.

Patrick, R., T. Bott R. Larson & C. Rhyne 1976. The Effect of Nitrilotriacetic Acid (NTA) on the Structure and Functioning of Aquatic Communities. Draft Report of Grant R801951 for Environmental Research Lab. Duluth, Minn. EPA (communicated to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders, Chicago, Ill., 1977).

Pickaver, A. H. 1974. The Production of N-nitrosoiminodiacetate from Sodium Nitrate and Nitrilotriacetic Acid, A Detergent Additive, By Microorganisms Isolated from Soil. Proc. Soc. Gen. Microbiol. 11: 5-6.

Pickaver, A. H. 1976. The Production of N-nitrosoiminodiacetate from Nitrilotriacetate and Nitrate by Microorganisms Growing in Mixed Culture. Soil Biol. Biochem. 8: 13-17.

Sagers, R. D. & I. C. Gunsalus 1961. Intermediary Metabolism of Diplococcus glycinophilus. I. Glycine Cleavage and One-Carbon Interconversions. J. Bact. 81: 541-549.

Stanier, R. Y., N. Palleroni & M. Doudoroff 1966. The Aerobic Pseudomonads: a Taxonomic Study. J. Gen. Microbiol. 43: 159-271.

Steenis, P. J., M. M. Cordes, J. G. H. Hilkens & F. Muller 1973. On the Interaction of para-Hydroxybenzoate Hydroxylase from Pseudomonas fluorescens with Halogen Ions. FEBS Letters 36: 177-180.



- Tabatabai, M. R. & Bremner, 1975. Decomposition of Nitrilotriacetate (NTA) in Soils. *Soil Biol. Biochem.* 7: 103-106.
- Tabor, C. W. & P. D. Kellogg 1970. Identification of Flavin Adenine Dinucleotide and Heme in a Homogeneous Spermidine Dehydrogenase from Serratia marcescens. *J. Biol. Chem.* 245: 5424-5433.
- Thompson, J. E. & J. R. Duthie 1968. The Biodegradability and Treatability of NTA. *J. Water Poll. Contr. Fed.* 40: 306-319.
- Thompson, J. E. & W. J. Jones, March 9, 1971. Procter and Gamble, unpublished information (cited in Monsanto/Procter & Gamble Report to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders, Cincinnati, Ohio, 1977).
- Tiedje, J. M. 1977. Communication to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders, Chicago, Ill.
- Tiedje, J. M. & B. B. Mason 1974. Biodegradation of Nitrilotriacetate (NTA) in Soils. *Soil Sci. Soc. Amer. Proc.* 38: 278-282.
- Tiedje, J. M., B. B. Mason, C. B. Warren, & E. J. Malec 1973. Metabolism of Nitrilotriacetate by Cells of a Pseudomonas Species. *Appl. Microbiol.* 25: 811-818.
- Warren, C. B. 1974. Biodegradation of Nitrilotriacetate Acid and NTA Metal Ion Complexes: The Chemo-biological Life Cycle of a Detergent Builder. *Survival in Toxic Environments*. Academic Press, N.Y. pp 473-496.
- Warren, C. B. & E. J. Malec 1972. Biodegradation of Nitrilotriacetic Acid and Related Imino and Amino Acids in River Water. *Science* 176: 277-279.
- Wong, P. T. S., D. Liu, & B. J. Dutka 1972. Rapid Biodegradation of NTA by a Novel Bacterial Mutant. *Water Res.* 6: 1577-1584.
- Wong, P. T. S., D. Liu, & D. J. McGirr 1973. Mechanism of NTA Degradation by a Bacterial Mutant. *Water Res.* 7: 1367-1374.
- Wright, R. 1975. Studies on Glycolic Acid Metabolism By freshwater Bacteria. *Limnol. Oceanogr.* 20: 626-633.



## NTA AND MUNICIPAL WASTEWATER MANAGEMENT

### REMOVAL OF NTA IN WASTEWATER TREATMENT

#### EFFECTIVENESS OF REMOVAL

Some reports of the efficiency of a variety of municipal wastewater treatment processes in removing NTA are summarized in Table I. Data on the fate of NTA in septic tank systems and in one sludge management process, anaerobic digestion, also are included. Table I is intended to concisely illustrate the nature of reported results. It is not an exhaustive summary--additional reports have been summarized in reviews prepared by Thom (1971), Prakash (1976), Thayer and Kensler (1973) and Toetz (1977).

In general, the results tabulated indicate that, under favourable conditions, acclimated aerobic biological wastewater treatment systems are capable of high degrees (say, 90 percent or more) of NTA removal. Low degrees of NTA removal are associated primarily with anaerobic conditions and low temperatures.

Anaerobic waste treatment processes included in Table I are the septic tanks, anaerobic sludge digesters, anaerobic septic tank tile systems and anaerobic soil systems. With a single exception, the data for these indicate inappreciable NTA removal. The exception was recently reported by Moore and Barth (1976). They found that, while no NTA removal occurred in anaerobic digestion of primary sewage sludge, good removal occurred when equal volumes of primary sludge and waste activated sludge containing NTA-acclimated organisms were anaerobically digested. While Moore and Barth's findings need to be expanded, available data indicate that NTA should be considered to move essentially unaffected through septic tanks, anaerobic sludge stabilization processes, anaerobic soils and any anaerobic systems adopted for treatment of municipal wastewaters (Young and McCarty, 1969).

Data on NTA removal in wastewater treatment systems are consistent in showing that removal efficiencies diminish as temperatures decrease. Closely controlled laboratory experiments with activated sludge, reported by Eden et al. (1972), showed NTA removal efficiencies above 90 percent when temperatures were above 10°C, but at 5°C, essentially no NTA removal occurred. Although the later conclusion is suspect because the 5°C system may not have acclimated during the 40 days, an acclimated system at 20°C, removing >98% of the added NTA, did drop in efficiency to 66 and 82% removal at 7.5°C for 20 mg/l and 5 mg/l of NTA respectively. BOD removed was unaffected by the temperature change. Other experiences reported in Table I are compatible with these findings. Because wastewater temperature is not a controlled variable in wastewater treatment, reduced NTA removal efficiencies from aerobic biological treatment



plants would be anticipated during cold seasons. Significant temperature reductions can occur in wastewater treatment processes such as stabilization ponds, extended aeration plants, aerated lagoons, and trickling filters, and such processes would be particularly susceptible to reduced NTA removal efficiency during cold weather.

Data on NTA removal from physical-chemical treatment processes such as adsorption and chemical precipitation and coagulation are absent from Table I. Extensive studies of NTA removal in physical-chemical treatment are not known, but in view of the purpose of NTA in detergent formulations, exceptionally high removal efficiencies would not be anticipated. Indeed, an OECD Committee (1973) indicated that NTA is not removed by chemical precipitation or adsorption on activated carbon.

Another process for which no data are included in Table I is effluent chlorination. Specific studies of the effects of wastewater chlorination practices on NTA are not known. Reactions of NTA and chlorine are reviewed elsewhere in this report. (Section 2)

#### MECHANISMS OF REMOVAL

Reductions in NTA concentrations which occur as wastewater flows through treatment plants are ordinarily assumed to be attributable to biological degradation. However, comprehensive studies of the exact removal mechanisms are not known. Elaboration of removal mechanisms is of concern because, if some removal involves incorporation of NTA into wastewater sludges, then the fate of the NTA in sludge becomes of concern. The question becomes particularly pertinent when it is considered that the most common type of sludge stabilization involves anaerobic treatment in which NTA biodegradation would not be expected. Furthermore, application of wastewater and stabilized sludge on agricultural land is a common practice, and questions concerning the effects of NTA in sludge and wastewater on heavy metals in soils would arise.

Swisher, *et al.* (1967) are commonly attributed with having demonstrated that NTA does not adsorb to activated sludge. Their results, however, merely indicated that the weight of NTA removed during a 30-day period exceeded the weight of sludge remaining in the system, and would not seem to preclude the possibility of association of NTA with sludge surfaces followed by biodegradation or removal from the process with sludge solids. In contrast with these findings Bouveng, *et al.*, (1968), who conducted specific tests to determine the amount of NTA adsorbed to activated sludge solids, found that an equilibrium was established between free and adsorbed NTA in activated sludge systems. At the beginning of batch feeding experiments, 25 and 36 percent of the NTA was reported to be adsorbed to sludge surfaces when 2 and 10 mg/l of NTA were added respectively.

Reported results of significant NTA removal in primary wastewater treatment are difficult to explain by biological mechanisms. In addition to the data on removal in primary sedimentation shown in Table I, Shumate, *et al.* (1970) found 8 percent NTA removal in primary sedimentation but attributed it to faulty sampling procedures. Biological oxidation seems unlikely because of the anoxic conditions which ordinarily prevail in primary sedimentation tanks and because of the lack of opportunity for developing an acclimated culture,



(except perhaps, as a result of wastage of activated sludge to primary sedimentation tanks).

Dunlap, et al. (1971) reported results of sorption studies with NTA and a variety of soils. They concluded that relatively weak sorptive forces were exhibited by these soils toward NTA but that the forces "cannot, however, be completely discounted in considering the movement of NTA into and through ground water". Studies of adsorption of NTA onto calcium carbonates, aluminum oxide, silica and kaolinite were reported by Huang and Ho (1977). They reported "significant adsorption of anionic NTA species... even for surfaces that are negatively or neutrally charged". Preliminary studies of NTA adsorption onto primary sludge and activated sludge carried out especially for this report by Gledhill (1977) showed only small amounts of NTA adsorption to the sludges at the end of a 30-min. contact period. With an initial NTA concentration of about 10 mg/l, maximum amounts of adsorption on activated sludge were about 0.24 mg/kg while the maximum amount on primary sludge was only about 0.008 mg/kg. Some adsorption studies with copper and nickel NTA chelates also were conducted with only small amounts of adsorption being reported during a 30-min. period.

To summarize, there remains a degree of uncertainty regarding the fate of NTA in wastewater treatment facilities. While it is commonly presumed that NTA does not reach anaerobic digesters in amounts greater than that contained in the liquid phase of the sludge, actual amounts of NTA in anaerobic digesters (and on possible subsequent agricultural land disposal sites) are apparently not known.

#### REMOVAL OF NTA COMPLEXES

Biodegradation of NTA/metal complexes is considered in more detail elsewhere in this report and only a few reports specific to wastewater treatment are mentioned here. In such studies, a question would seem to exist as to whether or not the NTA actually existed in the complexed form at the time experimental observations were made.

Swisher, et al. (1967) found no difference in the rate of biodegradation of NTA and a NTA-iron complex in laboratory activated sludge systems. Tiedje and Mason (1974), working in aerobic soil systems, found cadmium and mercury complexes of NTA to degrade more slowly than non-complexed NTA and, similarly, Shannon (1975) found that complexes with mercury, cadmium and nickel were resistant to degradation. Walker (1975) emphasized the need for considering all multivalent cations in the system. While NTA biodegradation was inhibited by copper or cadmium in his experiments the inhibitory effects were reduced by iron. Furthermore, increased hardness improved the biodegradation of the NTA in the presence of copper.

#### RELIABILITY OF NTA REMOVAL

While efficient NTA removal has been demonstrated in closely controlled acclimated aerobic biological wastewater treatment systems, it is prudent to consider the extent to which practical complications in real wastewater treatment systems would detract from that performance.



Appreciable periods of acclimation have commonly been reported to be required to achieve biological degradation of NTA. Thus, Swisher *et al.* (1967) found 2 to 3 weeks of acclimation to be required in laboratory activated sludge systems and Cleasby *et al.* (1974) needed 2 to 4 weeks to acclimate trickling filters to NTA. Klein found 5 to 7 weeks to be required in septic tank percolation fields. Renn (1974) tried in vain for a three-month period to acclimate an activated sludge system to NTA.

If NTA were adopted as a prevalent builder in detergent products, then its occurrence in most wastewaters would be continuous. Available data suggest that once acclimation was achieved, sustaining the acclimated condition would not be difficult unless overloading occurred. Pfeil and Lee (1968), for example, found that an acclimated culture recovered following two days without exposure to NTA, and Tiedje and Mason (1974) found that temporary exposure to anaerobic conditions did not severely harm an acclimated culture. Additionally, Cleasby *et al.* (1974) found that variations in NTA loading did not require an additional period of acclimation. However, when wastewater or sludge are applied to soil, application might be infrequent. Under such conditions acclimation to NTA might not be maintained.

The terms "refractory" or "biodegradable" as applied to biological wastewater treatment systems are relative terms. That is, a substance which is refractory at one mean cell residence time in a biological wastewater treatment process may be biodegradable at a longer mean cell residence time. Regrettably, insufficient kinetic data on NTA biodegradation appear to be available to permit estimation of loading conditions in biological wastewater treatment processes at which "washout" of organisms responsible for NTA biodegradation would occur. The fact that Bouveng *et al.* (1970) indicated that NTA biodegradation "dropped more than would be expected when the treatment plant was overloaded" suggests that there could be a reason for concern about the possibility that, during periods of overload, NTA degradation could be reduced more than that of other materials.

The adverse effect of low temperatures on NTA removal in biological wastewater treatment plants was reviewed in a previous section. It must be concluded with Eden *et al.* (1972) "that while NTA is almost completely removable under favorable conditions of sewage treatment, removal becomes incomplete under winter conditions". Eden *et al.*, (1972) elaborated further in emphasizing that their results were based on idealized laboratory conditions and that "it is probable that under actual working conditions at sewage works the removal during winter would be even lower".

As suggested by Eden *et al.*, it must be assumed that the practical problems of overloading, power failures, etc. which prevented Renn (1974) from achieving acclimation in a three-month period are not restricted to experimental studies, and that operational efficiencies below reported laboratory removal levels might be anticipated. Cleasby *et al.* (1974) felt that observed variability in NTA removal was caused by susceptibility of NTA-consuming species to random environmental changes, such as pH changes or the presence of toxic materials. In commenting on the removal of NTA under unfavorable circumstances in biological wastewater treatment plants, Bouveng *et al.* (1968) noted that "it seems justified to characterize it as a less preferred substrate".



The reported Canadian experience with NTA removal in full-scale facilities provides some measure of the extent to which practical considerations in real full-scale treatment plant operation can result in reduction of NTA removal efficiencies. Data summarized by Thayer and Kensler (1973) on reported experiences at a variety of Canadian wastewater treatment plants (included in Table I) are averages of monthly data for a six-month period terminating in May. During that period, seven different activated sludge plants averaged 72 percent removal of NTA, with one plant indicating an average removal efficiency of only 22 percent, and another 51 percent. The two trickling filter installations included in the tabulation indicated average NTA removals of only 34 percent.

Thus, it is apparent that if NTA is to be used as a detergent builder, it should be with the realization that efficient removal in present municipal wastewater treatment systems may not occur consistently. In acclimated aerobic biological treatment plants, effective removal under ideal conditions can take place, but those conditions may not always exist. Reduced efficiency of NTA removal is to be anticipated when low temperatures prevail. Available data from full-scale Canadian plants, and reported difficulties in achieving acclimation in wastewater treatment plants, suggest that practical problems of load variation, equipment maintenance, waste quality variation, etc. would cause further reduction in NTA removal efficiency.

## EFFECTS OF NTA ON WASTEWATER MANAGEMENT

### EFFECTS ON GENERAL PLANT PERFORMANCE

Municipal wastewaters contain a heterogeneous mixture of chemical compounds. It is unusual for a single compound to be as prevalent in wastewaters as NTA would be if it were used as a builder in detergents. In cases in which particular substances have been prevalent in municipal wastewaters (as, for example, when a particular industrial wastewater comprises a significant fraction of a total load on a treatment facility), changes in treatment plant performance attributable to those substances have not been unusual. Thus, it is appropriate to inquire as to the probable influence of NTA in wastewaters on factors such as sludge settleability, removal of carbonaceous and nitrogenous oxygen-demanding materials, phosphorus removal, and methane production.

A common effect of prevalent, unusual, constituents in wastewaters is to alter the settleability of biological sludges. Swisher *et al.* (1967) reported that NTA concentrations up to 200 mg/l did not alter activated sludge settleability. Similarly, Thompson and Duthie (1968) found no deleterious effects of NTA on activated sludge settleability. Thompson and Duthie also reported no effects on settleability of primary sludges. Cleasby *et al.* (1974) reported that, as measured by effluent suspended solid concentrations, NTA displayed no adverse effects on the settleability of trickling filter humus. No investigations on the effects of NTA, or of organisms synthesized in removal of NTA, on another important physical property of sludges, dewaterability, are known. However, in the absence of effects on settleability, significant interference with dewaterability would not be expected.

No effects of NTA on removal of oxygen demanding materials in activated sludge systems were noted by Shannon (1975) or by Swisher *et al.* (1967).



Similarly, Cleasby et al. (1974) reported no effects on removal of BOD, COD or TOC in trickling filters, and Klein (1974) reported that the presence of NTA had no noticeable effect on the performance of septic tank percolation fields or oxidation ponds.

Because of the question of increased metal ion toxicity in anaerobic digestion, it is of interest that Moore and Barth (1976), Thompson and Duthie (1968), and Klein (1974) all reported that NTA had no effects on this process.

The relationship between the presence of NTA and nitrification in a trickling filter plant were investigated by Cleasby et al. (1974). No adverse effects on nitrification were found.

No effect of NTA on biological removal of phosphate in an activated sludge wastewater treatment plant was recorded by Shannon (1975), but the average NTA concentration in the treatment plant influent was only 2.2 mg/l. No significant changes in phosphate removal when the concentration of NTA in the feed to a trickling filter was varied were reported by Cleasby et al. (1974).

Shannon (1975) reported that the reduction in wastewater phosphorus concentration brought about by substitution of NTA for a phosphate builder, more than compensated for any increase in dosage of alum or ferric chloride for phosphorus removal caused by the NTA. That is, lower dosages of alum and ferric chloride were required when NTA was used in place of a phosphorus-containing detergent. Because of the dependence of phosphorus removal by lime on pH, a comparable savings in lime dosage for phosphorus was not realized. Toetz (1977) cited work by Manning and Ramanoothy (1972) indicating that NTA may complex with calcium phosphate compounds and reduce the effectiveness of phosphorus precipitation by lime. Bouveng et al. (1968) concluded that the chelation of aluminum by NTA in alum precipitation of phosphorus was "of small practical significance".

#### TRANSPORT OF HEAVY METALS THROUGH WASTEWATER TREATMENT PLANT

Because of the ability of NTA to chelate heavy metals, the possibility of increases in heavy metal discharges from wastewater treatment plants as a result of NTA usage warrants investigation. In studies with trickling filters, Cleasby et al. (1974), found no significant metal transport as a result of the presence of NTA, except for manganese under low temperature conditions. Shannon (1975), however, found that with an influent NTA concentration of only 2.2 mg/l, zinc, aluminum, and copper transport through an activated sludge treatment plant increased under low temperature conditions. Similarly, Renn (1974) found that transport of zinc and iron through an activated sludge treatment plant increased as a result of NTA addition. When acclimation occurred, and NTA was removed in the treatment plant, the zinc concentration in the effluent decreased. Similarly, Nilsson (1971) indicated that heavy metals might be expected to be discharged from biological wastewater treatment plants in which NTA removal is incomplete because of overloading or low temperatures. Nilsson also cited work indicating that copper-NTA complexes and nickel-NTA complexes are not biologically degraded even under favorable conditions and will be discharged with biological wastewater treatment plant effluents.



In an evaluation of the effects of NTA on heavy metal removal in physical/chemical wastewater treatment plants, Argaman and Weddle (1974) concluded that "the data strongly indicate that NTA interferes significantly with the precipitation of specific heavy metals". Interference of NTA with precipitation of copper and cobalt with lime were particularly noted. Nilsson (1971) also indicated interference of NTA with heavy metal removal in chemical treatment processes, but indicated that the adverse effect was prevalent at pH values below 9.

#### EFFECTS ON LAND APPLICATION SYSTEMS

At least 20 percent of the sludge produced at wastewater treatment plants in the United States currently is applied to agricultural land, and increased application of sludge to land is anticipated in the future (Farrell, 1974). Such sludge is commonly subjected to anaerobic digestion prior to land application, but available data would indicate that NTA in the liquid phase of the sludge would not be completely degraded during digestion. As indicated in the previous section, the prevalence of NTA in the solid stage of the sludge is unknown. Additionally, application of wastewaters to land could be a source of NTA in soil systems. The amount of NTA applied in such cases would be dependent upon the type of and efficiency of wastewater treatment provided before land application. Because of the heavy metal chelation properties of NTA, the effects of these land application practices on heavy metal concentrations in ground water and on heavy metal introduction into the food chain through crop production requires analysis. As noted above the periodic use of land application is likely to make maintenance of an acclimated microbial population difficult.

#### OCEAN DISPOSAL

Another possible means for introduction of NTA into the environment from wastewater treatment processes is ocean disposal of wastewaters and sludges. Currently, 15 percent of the sludges produced in the United States are discharged to oceans (Farrell, 1974). Such sludges are commonly subjected to anaerobic digestion which would not be expected to eliminate whatever NTA might be present. Additionally, ocean discharges of wastewaters which had not been exposed to efficient aerobic biological treatment might still contain significant quantities of NTA.

Discharge of NTA to oceans is of potential concern because of the question (discussed elsewhere in this report) of biodegradation of NTA in certain marine environments. In the absence of such biodegradation, questions concerning the introduction of heavy metals into the marine food chain become pertinent.

#### EFFECTS OF NTA ON COSTS OF WASTEWATER MANAGEMENT

Analyses of the economic implications of NTA on the capital and operating costs for wastewater management are not known. Degradation of NTA in aerobic biological wastewater treatment processes would require oxygen and result in synthesis of microorganisms which would be wasted as sludge. The incremental costs of wastewater management attributable to the oxygen consumed and sludge produced in the course of NTA degradation depends on the relative amounts of NTA and biodegradable materials from other sources in wastewaters. For estimating purposes, it may be noted that the theoretical oxygen demand of NTA is 0.77 mg oxygen/mg NTA. It must be emphasized that builders which are alternatives to NTA also influence the cost of wastewater treatment.



## REFERENCES

- Argaman, Y. and C. L. Weddle, 1974. Fate of Heavy Metals in Physical-Chemical Treatment Processes. Water-1973. AIChE Symposium Series, 70: 136, 400-414.
- Bouveng, H. O., G. Davisson, and E. Steinberg, 1968. NTA in Sewage Treatment, 24: 348-359.
- Bouveng, H. O., P. Solyom and J. Werner, 1970. NTA in Sewage Treatment, Part 2. Degradation of NTA in a Trickling Filter and an Oxidation Pond. Vatten, 24: 389-402.
- Cleasby, J. L., D. W. Hubly, T. A. Ladd and E. A. Schon, 1974. Trickling Filtration of a Waste Containing NTA. Jour. Water Poll. Contr. Fed., 46: 1873-1887.
- Dunlap, W. J., R. L. Cosby, J. F. McNabb, B. E. Bledsoe, and M. R. Scalf, 1971. Investigation Concerning Probable Impact of Nitrilotriacetic Acid on Ground Waters. Environmental Water Pollution Control Research Series 16060 GHR 11/71, U.S. Environmental Protection Agency, Washington, D. C., 51 pp.
- Duthie, J. R., 1970. Trisodium Nitrilotriacetate (NTA) Environmental Safety Review. Procter and Gamble Company, Internal Report.
- Eden, G. E., G. E. Culley and R. C. Rootham, 1972. Effect of Temperature on the Removal of NTA (Nitrilotriacetic Acid) During Sewage Treatment. Water Research 6: 877-883.
- Farrell, J. B., 1974. Overview of Sludge Handling and Disposal. Proceedings National Conference on Municipal Sludge Management, Pittsburgh, Pennsylvania, June 11-13, 1974. Information Transfer, Incorporated, Washington, D. C., 5-10.
- Gledhill, W. E., 1977. NTA Adsorption to Primary Sludge and Activated Sludge. Monsanto Company, Internal Report, 5 pp.
- Huang, C. P. and A. Ho, 1977. Adsorption Characteristics of Nitrilotriacetate (NTA) at Solid Solution Interfaces. Manuscript submitted to Journal of Colloid and Interface Science, 16 pp.
- Klein, S. A., 1974. NTA Removal in Septic Tank and Oxidation Pond Systems. Jour. Water Poll. Contr. Fed., 46: 78-88.
- Manning, P. G. and S. Ramanoothy, 1972. Formation and Stability of Mixed-Ligand (NTA) and Phosphate Complexes of  $\text{Ca}^{++}$  and  $\text{Cu}^{++}$ . Inorganic Nuclear Chemistry Letters, 8: 653-658.
- Moore, L. and E. F. Barth, 1976. Degradation of NTA During Anaerobic Digestion, Jour. Water Poll. Contr. Fed., 48: 2406-2409.



Nilsson, R., 1971. Removal of Metals by Chemical Treatment of Municipal Waste Water. Water Research, 5: 51-60.

Organization for Economic Cooperation and Development, 1973. Report of the Expert Group on Detergents, OECD, Paris, 40 pp.

Pfeil, B. H. and G. F. Lee, 1968. Biodegradation of Nitrilotriacetic Acid in Aerobic Systems, Env. Sci. Tech., 2: 543-546.

Prakash, A., 1976. NTA (Nitrilotriacetic Acid) - An Ecological Appraisal. Environment Canada Water Pollution Control Directorate. Economic and Technical Review Report, EPS-3-WP-76-8.

Renn, C. E., 1974. Biodegradation of NTA Detergents in a Wastewater Treatment System. Jour. Water Poll. Contr. Fed., 46: 2363-2371.

Rudd, J. W. M., B. E. Townsend and R. D. Hamilton, 1973. Discharge of Nitrilotriacetate (NTA) from Two Sewage Treatment Facilities Located in a Midcontinental Climate. Jour. Fish. Res. Bd. Canada, 30: 1026-1030.

Shannon, E. E., 1975. Effect of Detergent Formulation on Wastewater Characteristics and Treatment. Jour. Water Poll. Contr. Fed., 47: 2371-2383.

Shumate, K. S., J. E. Thompson, J. D. Boakhart, and C. L. Dean, 1970. NTA Removal by Activated Sludge-Field Study. Jour. Water Poll. Contr. Fed., 42: 631-640.

Swisher, R. D., M. R. Crutchfield and D. W. Caldwell, 1967. Biodegradation of NTA in Activated Sludge, Env. Sci. and Technol, 1: 820-927.

Thayer, P. S. and C. J. Kensler, 1973. Current Status of the Environmental and Human Safety Aspects of Nitrilotriacetic Acid (NTA). CRC Critical Reviews in Environmental Control 3: 375-404.

Thom, N.S., 1971. Nitrilotriacetic Acid: A Literature Survey. Water Research, 5: 391-399.

Thompson, J. E. and J. R. Duthie, 1968. The Biodegradability and Treatability of NTA. Jour. Water Poll. Contr. Fed., 40: 306-319.

Tiedje, J. M. and B. B. Mason, 1974. Biodegradation of Nitrilotriacetate (NTA) in Soils. Soil Sci. Soc. Amer. Proc., 38: 278-282.

Toetz, D. W., 1977. Fate, Transport and Effects of NTA in the Aquatic Environment, Draft Report. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia, 72 pp.

Young, J. C. and P. L. McCarty, 1969. The Anaerobic Filter for Waste Treatment, Jour. Water Poll. Contr. Fed., Res. Suppl., 41: R160-R173.

Walker, A. P., 1975. Ultimate Biodegradation of Nitrilotriacetate in the Presence of Heavy Metals, Progress in Water Technology, 7: 555-560.



TABLE I

## REPORTED EFFICIENCIES OF NTA REMOVAL IN MUNICIPAL WASTEWATER TREATMENT FACILITIES

Waste Treatment Processes	Influent NTA Concentration, (mg/l)	NTA Removal Efficiency (percent)	Notes	Reference
Primary Sedimentation	4.8	30	Average for 6-month period from Canadian City	Thayer and Kensler, 1973
	2.0	58	Average for 6-month period from Canadian City	Thayer and Kensler, 1973
	1.6	11	Average for 6-month period from Canadian City	Thayer and Kensler, 1973
Activated Sludge	20	92	Laboratory continuous flow units	Swisher <u>et al.</u> , 1967
	5 and 20	3*	Laboratory Studies at 5°C	Eden <u>et al.</u> , 1972
	5 and 20	66-82	Laboratory Studies at 7.5°C	Eden <u>et al.</u> , 1972
	5 and 20	98	Laboratory Studies at 20°C	Eden <u>et al.</u> , 1972
	--	High	Full scale-effluent < 0.5 mg NTA/l in winter and summer	Rudd, <u>et al.</u> , 1973
	10	90	Laboratory Scale	Thompson and Duthie, 1968
	2.2	61	Full Scale, at 14°C	Shannon, 1975
	5 to 45	65 to 98	Full Scale	Renn, 1974
	10	80 to 85	Laboratory Units at 20°C	Bouveng <u>et al.</u> , 1968
	10	25	Laboratory Units at 5°C	Bouveng <u>et al.</u> , 1968

\*Possibly unacclimated



TABLE I (CONTINUED)

Waste Treatment Processes	Influent NTA Concentration, (mg/l)	NTA Removal Efficiency (percent)	Notes	Reference
	up to 110	essentially total	Laboratory Units	Pfeil and Lee, 1968
	2.2 to 5.1	11 to 96	Range of averages for monthly data for 6-month period from 7 Canadian Cities. Average removal = 72 percent	Thayer and Kensler, 1973
	up to 8	90	Full Scale	Shumate <u>et al.</u> , 1970
	16	75	Full Scale	Shumate <u>et al.</u> , 1970
Trickling Filter	--	over 80	Full Scale data-summer and early autumn. Efficiency dropped when plant overloaded or temperatures low.	
	16	90	Pilot scale-fall conditions	Cleasby <u>et al.</u> , 1974
	8	75	Pilot scale-cold weather, but filter heated	Cleasby <u>et al.</u> , 1974
	1.3	32	Average for 6-month period from Canadian City	Thayer and Kensler, 1973
	4.5	36	Average for 6-month period from Canadian City	Thayer and Kensler, 1973
Aerated Lagoon	--	high	Full scale summer	Rudd <u>et al.</u> , 1973
	--	essentially zero	Full scale winter	Rudd <u>et al.</u> , 1973



TABLE I (CONTINUED)

Waste Treatment Processes	Influent NTA Concentration, (mg/l)	NTA Removal Efficiency (percent)	Notes	Reference
Stabilization Pond	up to 30	90	360 gal pilot scale-30 day retention	Klein, 1974
	up to 75	80	360 gal pilot scale-30 day retention	Klein, 1974
	100	50	360 gal pilot scale-30 day retention	Klein, 1974
	--	near 100	Full scale-summer	Bouveng <i>et al.</i> , 1970
	--	low	Full scale-winter	Bouveng <i>et al.</i> , 1970
Anaerobic Digestion	15 to 60	8 to 14	4 l laboratory units-37°C, 30-day retention time	Klein, 1974
	10 to 20	0	3 l laboratory units-35°C, 30-day retention time, with primary sludge feed	Moore and Barth, 1976
	20	near 100	3 l laboratory units-35°C, 30-day retention time, with 50% primary sludge and 50% by volume waste activated sludge	Moore and Barth, 1976
Septic Tank	61	27	Laboratory 127 l septic tank	Klein, 1974
	31	26	Laboratory 127 l septic tank	Klein, 1974
Septic Tank Tile Fields	up to 60	essentially 100	Aerobic, but with dissolved oxygen concentrations as low as 0.5 mg/l	Klein, 1974
	50	10	Anaerobic	Klein, 1974



TABLE I (CONTINUED)

Waste Treatment Processes	Influent NTA Concentration, (mg/l)	NTA Removal Efficiency (percent)	Notes	Reference
Soil Systems	40 to 100	95	Aerobic-Yolo sandy loam in laboratory column	Klein, 1974
	10 to 100	10 to 15	Anaerobic-Yolo sandy loam laboratory column	Klein, 1974
	40	"total"	Aerobic-good removal at NTA concentrations as high as 600 mg/l	Tiedje and Mason, 1974
	40	0	Anaerobic-also, degradation limited under microaerophilic conditions	Tiedje and Mason, 1974
	50	essentially complete	Aerobic	Dunlap <u>et al.</u> , 1971
	50	essentially none	Anaerobic	Dunlap <u>et al.</u> , 1971



# TABLE 1

Depth (meters)	Date	Time	Wind direction	Wind speed (knots)	Wave height (meters)	Water temperature (°C)	Air temperature (°C)	Relative humidity (%)	Barometric pressure (mm Hg)	Remarks	Date
0	1971	10:00	030	10	1.5	25.0	28.0	75	1010.0	Clear sky	1971
10	1971	10:05	030	10	1.5	24.5	27.5	75	1010.0	Clear sky	1971
20	1971	10:10	030	10	1.5	24.0	27.0	75	1010.0	Clear sky	1971
30	1971	10:15	030	10	1.5	23.5	26.5	75	1010.0	Clear sky	1971
40	1971	10:20	030	10	1.5	23.0	26.0	75	1010.0	Clear sky	1971
50	1971	10:25	030	10	1.5	22.5	25.5	75	1010.0	Clear sky	1971
60	1971	10:30	030	10	1.5	22.0	25.0	75	1010.0	Clear sky	1971
70	1971	10:35	030	10	1.5	21.5	24.5	75	1010.0	Clear sky	1971
80	1971	10:40	030	10	1.5	21.0	24.0	75	1010.0	Clear sky	1971
90	1971	10:45	030	10	1.5	20.5	23.5	75	1010.0	Clear sky	1971
100	1971	10:50	030	10	1.5	20.0	23.0	75	1010.0	Clear sky	1971
110	1971	10:55	030	10	1.5	19.5	22.5	75	1010.0	Clear sky	1971
120	1971	11:00	030	10	1.5	19.0	22.0	75	1010.0	Clear sky	1971
130	1971	11:05	030	10	1.5	18.5	21.5	75	1010.0	Clear sky	1971
140	1971	11:10	030	10	1.5	18.0	21.0	75	1010.0	Clear sky	1971
150	1971	11:15	030	10	1.5	17.5	20.5	75	1010.0	Clear sky	1971
160	1971	11:20	030	10	1.5	17.0	20.0	75	1010.0	Clear sky	1971
170	1971	11:25	030	10	1.5	16.5	19.5	75	1010.0	Clear sky	1971
180	1971	11:30	030	10	1.5	16.0	19.0	75	1010.0	Clear sky	1971
190	1971	11:35	030	10	1.5	15.5	18.5	75	1010.0	Clear sky	1971
200	1971	11:40	030	10	1.5	15.0	18.0	75	1010.0	Clear sky	1971
210	1971	11:45	030	10	1.5	14.5	17.5	75	1010.0	Clear sky	1971
220	1971	11:50	030	10	1.5	14.0	17.0	75	1010.0	Clear sky	1971
230	1971	11:55	030	10	1.5	13.5	16.5	75	1010.0	Clear sky	1971
240	1971	12:00	030	10	1.5	13.0	16.0	75	1010.0	Clear sky	1971
250	1971	12:05	030	10	1.5	12.5	15.5	75	1010.0	Clear sky	1971
260	1971	12:10	030	10	1.5	12.0	15.0	75	1010.0	Clear sky	1971
270	1971	12:15	030	10	1.5	11.5	14.5	75	1010.0	Clear sky	1971
280	1971	12:20	030	10	1.5	11.0	14.0	75	1010.0	Clear sky	1971
290	1971	12:25	030	10	1.5	10.5	13.5	75	1010.0	Clear sky	1971
300	1971	12:30	030	10	1.5	10.0	13.0	75	1010.0	Clear sky	1971
310	1971	12:35	030	10	1.5	9.5	12.5	75	1010.0	Clear sky	1971
320	1971	12:40	030	10	1.5	9.0	12.0	75	1010.0	Clear sky	1971
330	1971	12:45	030	10	1.5	8.5	11.5	75	1010.0	Clear sky	1971
340	1971	12:50	030	10	1.5	8.0	11.0	75	1010.0	Clear sky	1971
350	1971	12:55	030	10	1.5	7.5	10.5	75	1010.0	Clear sky	1971
360	1971	13:00	030	10	1.5	7.0	10.0	75	1010.0	Clear sky	1971
370	1971	13:05	030	10	1.5	6.5	9.5	75	1010.0	Clear sky	1971
380	1971	13:10	030	10	1.5	6.0	9.0	75	1010.0	Clear sky	1971
390	1971	13:15	030	10	1.5	5.5	8.5	75	1010.0	Clear sky	1971
400	1971	13:20	030	10	1.5	5.0	8.0	75	1010.0	Clear sky	1971
410	1971	13:25	030	10	1.5	4.5	7.5	75	1010.0	Clear sky	1971
420	1971	13:30	030	10	1.5	4.0	7.0	75	1010.0	Clear sky	1971
430	1971	13:35	030	10	1.5	3.5	6.5	75	1010.0	Clear sky	1971
440	1971	13:40	030	10	1.5	3.0	6.0	75	1010.0	Clear sky	1971
450	1971	13:45	030	10	1.5	2.5	5.5	75	1010.0	Clear sky	1971
460	1971	13:50	030	10	1.5	2.0	5.0	75	1010.0	Clear sky	1971
470	1971	13:55	030	10	1.5	1.5	4.5	75	1010.0	Clear sky	1971
480	1971	14:00	030	10	1.5	1.0	4.0	75	1010.0	Clear sky	1971
490	1971	14:05	030	10	1.5	0.5	3.5	75	1010.0	Clear sky	1971
500	1971	14:10	030	10	1.5	0.0	3.0	75	1010.0	Clear sky	1971
510	1971	14:15	030	10	1.5	-0.5	2.5	75	1010.0	Clear sky	1971
520	1971	14:20	030	10	1.5	-1.0	2.0	75	1010.0	Clear sky	1971
530	1971	14:25	030	10	1.5	-1.5	1.5	75	1010.0	Clear sky	1971
540	1971	14:30	030	10	1.5	-2.0	1.0	75	1010.0	Clear sky	1971
550	1971	14:35	030	10	1.5	-2.5	0.5	75	1010.0	Clear sky	1971
560	1971	14:40	030	10	1.5	-3.0	0.0	75	1010.0	Clear sky	1971
570	1971	14:45	030	10	1.5	-3.5	-0.5	75	1010.0	Clear sky	1971
580	1971	14:50	030	10	1.5	-4.0	-1.0	75	1010.0	Clear sky	1971
590	1971	14:55	030	10	1.5	-4.5	-1.5	75	1010.0	Clear sky	1971
600	1971	15:00	030	10	1.5	-5.0	-2.0	75	1010.0	Clear sky	1971
610	1971	15:05	030	10	1.5	-5.5	-2.5	75	1010.0	Clear sky	1971
620	1971	15:10	030	10	1.5	-6.0	-3.0	75	1010.0	Clear sky	1971
630	1971	15:15	030	10	1.5	-6.5	-3.5	75	1010.0	Clear sky	1971
640	1971	15:20	030	10	1.5	-7.0	-4.0	75	1010.0	Clear sky	1971
650	1971	15:25	030	10	1.5	-7.5	-4.5	75	1010.0	Clear sky	1971
660	1971	15:30	030	10	1.5	-8.0	-5.0	75	1010.0	Clear sky	1971
670	1971	15:35	030	10	1.5	-8.5	-5.5	75	1010.0	Clear sky	1971
680	1971	15:40	030	10	1.5	-9.0	-6.0	75	1010.0	Clear sky	1971
690	1971	15:45	030	10	1.5	-9.5	-6.5	75	1010.0	Clear sky	1971
700	1971	15:50	030	10	1.5	-10.0	-7.0	75	1010.0	Clear sky	1971
710	1971	15:55	030	10	1.5	-10.5	-7.5	75	1010.0	Clear sky	1971
720	1971	16:00	030	10	1.5	-11.0	-8.0	75	1010.0	Clear sky	1971
730	1971	16:05	030	10	1.5	-11.5	-8.5	75	1010.0	Clear sky	1971
740	1971	16:10	030	10	1.5	-12.0	-9.0	75	1010.0	Clear sky	1971
750	1971	16:15	030	10	1.5	-12.5	-9.5	75	1010.0	Clear sky	1971
760	1971	16:20	030	10	1.5	-13.0	-10.0	75	1010.0	Clear sky	1971
770	1971	16:25	030	10	1.5	-13.5	-10.5	75	1010.0	Clear sky	1971
780	1971	16:30	030	10	1.5	-14.0	-11.0	75	1010.0	Clear sky	1971
790	1971	16:35	030	10	1.5	-14.5	-11.5	75	1010.0	Clear sky	1971
800	1971	16:40	030	10	1.5	-15.0	-12.0	75	1010.0	Clear sky	1971
810	1971	16:45	030	10	1.5	-15.5	-12.5	75	1010.0	Clear sky	1971
820	1971	16:50	030	10	1.5	-16.0	-13.0	75	1010.0	Clear sky	1971
830	1971	16:55	030	10	1.5	-16.5	-13.5	75	1010.0	Clear sky	1971
840	1971	17:00	030	10	1.5	-17.0	-14.0	75	1010.0	Clear sky	1971
850	1971	17:05	030	10	1.5	-17.5	-14.5	75	1010.0	Clear sky	1971
860	1971	17:10	030	10	1.5	-18.0	-15.0	75	1010.0	Clear sky	1971
870	1971	17:15	030	10	1.5	-18.5	-15.5	75	1010.0	Clear sky	1971
880	1971	17:20	030	10	1.5	-19.0	-16.0	75	1010.0	Clear sky	1971
890	1971	17:25	030	10	1.5	-19.5	-16.5	75	1010.0	Clear sky	1971
900	1971	17:30	030	10	1.5	-20.0	-17.0	75	1010.0	Clear sky	1971
910	1971	17:35	030	10	1.5	-20.5	-17.5	75	1010.0	Clear sky	1971
920	1971	17:40	030	10	1.5	-21.0	-18.0	75	1010.0	Clear sky	1971
930	1971	17:45	030	10	1.5	-21.5	-18.5	75	1010.0	Clear sky	1971
940	1971	17:50	030	10	1.5	-22.0	-19.0	75	1010.0	Clear sky	1971
950	1971	17:55	030	10	1.5	-22.5	-19.5	75	1010.0	Clear sky	1971
960	1971	18:00	030	10	1.5	-23.0	-20.0	75	1010.0	Clear sky	1971
970	1971	18:05	030	10	1.5	-23.5	-20.5	75	1010.0	Clear sky	1971
980	1971	18:10	030	10	1.5	-24.0	-21.0	75	1010.0	Clear sky	1971
990	1971	18:15	030	10	1.5	-24.5	-21.5	75	1010.0	Clear sky	1971
1000	1971	18:20	030	10	1.5	-25.0	-22.0	75	1010.0	Clear sky	1971

TABLE 1 (CONTINUED)



# DEGRADATION OF NTA IN THE ENVIRONMENT

## AEROBIC DEGRADATION

### FRESH WATER

Significant degradation of NTA can occur in fresh waters such as streams, rivers, and lakes where NTA enters in untreated wastewaters or by incomplete removal in low efficiency and overloaded treatment plants. Warren & Malec (1972) observed, after a 6 week lag, essentially complete degradation of NTA in the Detroit and Meramec Rivers at 6°C with no detectable intermediates accumulated. When NTA levels were monitored in Grindstone Creek, a stream receiving wastewater from the Waterdown, Ontario, treatment plant, biodegradation and dilution gave NTA concentrations in the summer months (16-21°C) consistently less than 10 µg/l under different conditions of treatment plant loading (Shannon et al., 1974). At temperatures of 0.5-3°C efficiency of NTA removal in the plant was reduced from >95% to <45% giving stream concentrations as high as 125 µg/l. Despite the lower temperatures subsequent NTA disappearance was somewhat greater than could be accounted for by estimates of the dilution factor. Laboratory studies with water and microorganisms from Grindstone Creek showed, in fact, that biodegradation of NTA could occur and was slowed by decreasing the temperature of incubation (approximately two fold for each decrement of 10 C).

Laboratory microcosms also accomplish NTA degradation after acclimation (2 weeks) at rates which are directly proportional to the concentration of NTA (Bott et al., 1976; Bott, 1977). By contrast Ferguson et al. (1971) observed removal of less than 1 mg/l when NTA was added to experimental streams at an influent concentration of 10 mg/l.

Chau and Shiomi (1972) have shown that NTA is readily degraded in lakes. Concentrations of NTA added at 1, 5 and 10 mg/l to stirred lakewater samples disappeared completely in periods from 5-11 days; after a second addition of NTA degradation was complete in 3-4 days. Formation of nitrate was also noted. Under winter conditions NTA is effectively degraded albeit more slowly (Rudd et al., 1973; Matheson, 1977).

### SALT WATERS

Bacteria have been isolated from seawater capable of growth with NTA as sole nitrogen or nitrogen and carbon source in a synthetic estuarine medium (Erikson et al., 1970). NTA utilization in this system was poor. Gledhill and McDonald (1976) have followed the degradation of <sup>14</sup>C-labelled NTA in model marine and estuarine systems. While 97.5% of NTA was removed from the marine system by the sixth week, no significant removal of NTA occurred in estuarine systems by the 12th week. Attempts to isolate from an estuary organisms capable



of degrading NTA in estuarine environments were unsuccessful (Bourquin & Przybyszewski, 1977) whereas such environments yielded organisms capable of degrading NTA in fresh water. Results with the Pseudomonas strain isolated by Tiedje (Tiedje et al., 1973) established that this organism degraded NTA at reduced rates in saline environments. Apparently some factor in estuarine environments ( $\text{Na}^+$ , Cl or ionic strength?) has an inhibitory effect upon reactions of NTA degradation in fresh water organisms. No such effect is apparent for marine bacteria able to degrade NTA. Effects of chloride and other anions on purified monooxygenases have been described (see earlier sections). Further studies are required to explain the special circumstances in estuarine environments affecting NTA-degradation.

## SOIL

Studies by Tiedje and Mason with  $^{14}\text{C}$ -NTA (Tiedje & Mason, 1974) have shown that NTA undergoes conversion to  $\text{CO}_2$  in most soils examined over a wide range of concentrations (10-600 ppm) either immediately or after a lag of up to 6 days. Different patterns of degradation suggested that in some soils degradation was effected by NTA-utilizers while in others partial degradation might be accomplished by non-utilizers. Degradation occurred only in the presence of oxygen; while rates of NTA oxidation were similar with air and with 1.0%  $\text{O}_2$ :99%  $\text{N}_2$ , reduced oxidation rates were seen at 0.1%  $\text{O}_2$ , and were essentially absent under an argon atmosphere. Degradation was significant at  $24^\circ\text{C}$  and  $12.5^\circ\text{C}$  but negligible at  $2^\circ\text{C}$ . After a period of acclimatization at  $12.5^\circ\text{C}$  rates of degradation at this temperature were more rapid and measurable at  $2^\circ$  suggesting a shift in the bacterial population from mesophilic to psychrophilic forms. In the soils studied by Tabatabai & Bremner (1975), degradation of NTA proceeded at similar rates under aerobic and anaerobic conditions. Differences in their findings from those of Tiedje and Mason (1974) may lie in the different methods used to measure NTA removal. Interestingly the formation of  $\text{NO}_3$  in aerobic soils indicates that NTA at the concentrations used does not interfere with soil nitrification.

## ANAEROBIC DEGRADATION

### SOILS

In their studies of the breakdown of NTA by soil microflora Tiedje and Mason (1974) found that no  $\text{CO}_2$  was formed from NTA under anaerobic conditions. By contrast NTA was shown to disappear from different soils at similar rates under aerobic and anaerobic conditions by Tabatabai & Bremner, (1975). Dunlap et al., (1971) found that after a suitable acclimation period, NTA at 50 mg/l was completely and rapidly removed from sand, loam and clay loam in percolation columns operated under aerobic or unsaturated conditions. Under water-logged or saturated conditions, simulating flooded percolation fields serving septic tanks, the same columns effected a greatly decreased removal of NTA, indicating the possibility that NTA could enter ground waters under these conditions and even solubilize and transport metal ions from such soils. Similar findings with simulated percolation fields were reported by Klein (1974) where flooding reduced dissolved oxygen and also the extent of NTA removal from 100% to 15-35%. Even then some of this latter removal was attributed to residual dissolved oxygen.



## GROUND WATER

In an attempt to investigate the anaerobic degradation of NTA in ground water, Dunlap et al. (1971) constructed simulated aquifers. Disappearance of NTA from 30-32 mg/l to essentially zero was observed in a period of 52 days at 20°C. Anaerobic conditions were confirmed by the demonstration of methane formation; when  $^{14}\text{C}$ -NTA was used the methane formed contained radioisotope. Consequently any NTA reaching the water table unchanged by virtue of extensive flooding of a percolation field apparently can undergo anaerobic degradation in aquifers.

## LAKE HYPOLIMNIA

The effect of anoxic conditions on NTA degradation in natural waters has formed the basis of a study by Patrick et al. (1976). Anaerobic conditions did not prevent NTA degradation but retarded the process relative to its aerobic disappearance.

Prakash (1976) cites a personal communication from R.D. Hamilton, Freshwater Institute, Winnipeg, concerning in situ studies of NTA degradation in small lakes (see also Hamilton, 1977). Apparently transient accumulation of NTA in the hypolimnion can result from its constant input during thermal stratification in summer months. Only during fall overturn could NTA decrease by degradation and dilution. With continued NTA loading in late fall and winter NTA could again accumulate because of slower rates of degradation and poor mixing and could persist until the spring overturn.

## FACTORS INVOLVED IN NTA DEGRADATION

### ACCLIMATION - A SPECULATION

In laboratory and field studies of the aerobic and anaerobic degradation of NTA, periods of acclimation are frequently necessary before NTA removal occurs at significant rates (Swisher et al., 1967; Shumate et al., 1970; Cleasby et al., 1974; Thompson and Duthie, 1968; Pfeil & Lee, 1968; Bouveng et al., 1968; Patrick et al., 1976). Once acclimated, populations of bacteria generally retain the properties for which they have been selected. Often lag times as long as several weeks or months are needed before useful degrading populations are established. Suggestions concerning enzyme induction and increases in original small subpopulations are inadequate to account for the long lags observed. For NTA, which is not a natural product, degradation is only possible if some organism possesses an enzyme with a sufficiently broad specificity to attack it in a useful manner, and which is already present at sufficient levels within the cell to accomplish a significant degree of conversion. Lacking these prerequisites evolutionary steps are needed, such as mutations in regulatory genes (spontaneous or induced) to give appropriate amounts of enzyme; mutations in structural genes may be needed to develop or alter enzyme specificity in a suitable manner. Such evolution of new functions relies on recruitment of some preexisting enzyme which may be elaborated by only a small fraction of the total microbial flora. According to this theory long lag periods may be needed because the necessary spontaneous mutations are random and many are disadvantageous.



Evolution of NTA-degrading organisms by enzyme recruitment can be seen to depend on a preexisting capacity to perform a reaction which can be related to NTA. The performance of the specifically resulting acclimated bacterial population is of course still subject to changes in substrate concentration (NTA and oxygen), temperature and other factors.

Acclimation of bacterial populations to lowered temperatures is necessary for the rapid biodegradation of all organic compounds by virtue of the general temperature optima for different microbial groups. A shift from 25°C to 5°C generally requires the substitution of a psychrophilic population for a mesophilic one. Without such population shifts rates of degradation of organic compounds will show pronounced dependence upon ambient temperature. Seasonal changes in temperature require such population shifts in treatment plants and elsewhere; for diverse microbial flora capable of degrading a wide variety of commonplace organic compounds such shifts can select appropriate organisms rapidly from large and varied populations. The population of NTA-degrading organisms, established possibly by evolution, is unlikely to be so varied; a drop in temperature will place a selection pressure on a less diverse population which may not develop the appropriate psychrophilic character readily. A separate evolutionary step from a diverse psychrophilic population may be the easier selection process. Such limitations can be perceived as important factors in the degradation of organic compounds by bacterial populations lacking a high degree of heterogeneity. In natural waters where microbial numbers are considerably lower than in treatment plants these limitations are amplified even further.

It should be stressed, therefore, that less diverse microbial populations are more vulnerable to environmental change than are complex populations, and that acclimation once achieved under one set of conditions may not be so readily adapted to others.

#### TEMPERATURE

Degradation of NTA by microorganisms has been shown at temperatures of 2-30°C. At temperatures of 10°C and below, the rate of its degradation in treatment facilities is substantially decreased. For example, at 7.5°C rates can be reduced to values as low as 66-82% of those at 20°C (Eden *et al.*, 1972; Rudd & Hamilton, 1972). Selection from soil of microbial populations with improved abilities to degrade NTA at low temperatures can occur, however, (Tiedje & Mason, 1974). Slower rates of degradation at lowered temperatures will be most pronounced where NTA degradation is already slow because of its chelation to certain metals. (See below).

#### DEGRADATION OF NTA-CHELATES

A major concern about degradation of NTA in different environments is directly related to that property which makes it most useful, *viz.* what is the extent to which its biodegradation is impaired by its chelation to different metal ions? Numerous studies have been conducted on the degradation of different chelates at varying concentrations in environments as diverse as fresh water, (Björndal *et al.*, 1972; Swisher *et al.*, 1973; Shannon, 1975; Dunlap *et al.*, 1971; Chau & Shiomi, 1972); soils, (Tiedje & Mason, 1974; Dunlap *et al.*, 1971);



and activated sludge, (Björndal *et al.*, 1972; Forsberg & Lindquist, 1967; Swisher *et al.*, 1967; Walker, 1975; Gudernatsch, 1970, 1975; Huber & Popp, 1972). Warren (1974) has summarized many of the results of this work. Of the various metal chelates tested, those of NTA with Cu, Cd, Ni, and Zn are generally removed at noticeably slower rates than others such as Ca, Mg, Fe, and Pb. Slower rates also observed with the Hg chelate may be more directly related to the general toxic effect of Hg ions. An incomplete attack on, say, the Cu-NTA complex may be related to a similar effect - the toxicity of Cu ions released from the chelate as degradation proceeds.

When one considers the various metal chelates formed by NTA at environmentally realistic levels, it can be appreciated that in natural environments the most readily degradable chelates will disappear first resulting in a redistribution of chelant to accommodate released ions. In other words, NTA degradation will be funnelled through the more readily degraded complexes. At low NTA concentrations the complexes which NTA will form depend upon the concentrations of metal ions available and the values of the dissociation constants for each of these species at the pH considered. The distribution of metals complexed to NTA at a concentration of 25 ppb in a typical river water will be 52% Cu, 34% Ni, 9% Ca and 5% Zn (International Joint Commission, 1977).

Chelates of NTA with Mg, Ca, Mn, Ba, Sr, and Fe are apparently tridentate complexes with lower dissociation constants than the tetradentate complexes formed with Cu, Ni, Co, Zn, Cd, and Pb (Warren, 1974). It is fortunate, therefore, that calcium salts are present at such high concentrations in many bodies of water, since this results in much of the NTA being present in a complex in which it is readily degradable.

It is for this reason that the addition of Ca or the presence of high levels of water hardness can facilitate degradation of the more persistent chelates of NTA with Cd (Huber & Popp, 1972), and Cu (Björndal, 1972). For the same reason ion exchange processes with Fe- and Ca- containing soils and sediments can lead to increases in that fraction of NTA present as its tridentate chelates. Ligand exchange can also lead to a more favorable distribution of NTA in its tridentate complexes. Thus other organic ligands can compete for various metal ions; sewage sludge for example can adsorb Cu more readily than Cd or Ni (Björndal *et al.*, 1972). Under environmental conditions, therefore, NTA-chelates undergo metal ion exchange and ligand exchange reactions in solution which facilitate degradation of NTA.

Another concern relating to the chelating properties of NTA is that it may solubilize toxic metals from such locations as lake sediments and soil, and then release these metals in a soluble form as the NTA portion of the complex is degraded. While it can be shown that NTA is capable of solubilizing different metals from sediments (Chau & Shiomi, 1972; Taylor *et al.*, 1972; Gregor, 1972) and of mobilizing metal ions in sand and soil columns (Dunlap *et al.*, 1971) the concentrations of NTA used in such studies (from 1-100 mg/l) are higher than might realistically be anticipated to be present after waste treatment. Furthermore it has been shown by Chau & Shiomi (1972) that as NTA is broken down by the



lake microflora, the earlier solubilized metal ions no longer remain soluble. Factors such as water hardness, the metals to which NTA is chelated, and the presence of other organic ligands such as humic acids, and amino acids will all influence the qualitative and quantitative aspects of solubilization, but once NTA is degraded, its solubilizing effect is removed, and metal ions apparently return to their former insoluble state as determined by the solubility products of their salts. The presence of the more persistent metal chelates, in anaerobic environments, at low temperatures, where dissolved organics and exchangeable minerals are absent, and where there is no water hardness, represents a scenario for minimal complex degradation. In the unlikely event that all the above conditions are satisfied the problem is only a temporal one since water movement will lead to alterations in one or more of these factors, favoring increasing complex degradation.

Lastly, it should be mentioned that NTA has certain beneficial effects upon degradative processes. Metal ions such as Hg, Cd, Cu, Ni and Zn can interfere with the degradation of linear alkyl benzene sulfonates in river water. The addition of NTA at 5 mg/l reduces this interference in all cases except that caused by Hg (Swisher et al., 1973).

Inhibitory effects of metal ions on respiration and glucose utilization by stream microflora were also reduced or prevented by addition of NTA (Patrick et al., 1976).

## REFERENCES

- Björndal, H., H. O. Bouveng, P. Solyom & J. Werner 1972. NTA in treatment. Part 3. Biochemical Stability of Some Metal Chelates. *Vatten* 28(1): 5-16.
- Bott, T. L., J. Preslan, J. Finlay, & R. Brunner 1976. Use of Flowing Water Microcosms and Ecosystem Streams to Study Microbial Degradation of Leaf Litter and Nitrilotriacetic Acid. Paper presented at 1976 SIM Meeting.
- Bott T. L. 1977. Communication to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders. Chicago, 1977.
- Bourquin, A. W. & V. A. Przybyszewski 1976. Distribution of Bacteria with Nitrilotriacetate-Degrading Potential in an Estuarine Environment. Submitted for publication. Prepublication Presented to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders, Chicago, 1977.
- Bouveng, H. O., G. Davisson, & E. M. Steinberg 1968. NTA in Sewage Treatment. *Vatten* 24: 348-359.
- Chau, Y. K. and M. T. Shiomi 1972. Complexing Properties of Nitrilotriacetic acid in the Lake Environment. *Water, Air and Soil Pollution*. Dordrecht 1: 149-164.
- Cleasby, J. L., D. W. Hubly, T. A. Ladd & E. A. Schon 1974. Treatment of Waste Containing NTA in a Trickling Filter. *J. Water Poll. Cont. Fed.* 46: 1873-1887.



Dunlap, W. J., R. L. Crosby, J. F. McNabb, B. E. Bledsoe, and M. R. Scalf. 1971. Investigation concerning probable impact of nitrilotriacetic acid on ground waters. Environmental Protection Agency, Ada, Oklahoma. U.S. Gov. Printing Office, Washington, D.C. Project #16060, Nov. 1971. pp. 55.

Eden, G. E., G. E. Culley, & R. C. Rootham 1972. Effect of Temperature on the Removal of NTA (nitrilotriacetic acid) During Sewage Treatment. Water Res. 6: 877-883.

Erickson, S. J., T. E. Maloney, and J. H. Gentile 1970. Effect of Nitrilotriacetic Acid on the Growth and Metabolism of Estuarine Phytoplankton. J. Water Poll. Cont. Fed. 42: R329-R335.

Ferguson, B., R. Todd, H. Holm, \*J. Pope, & H. Kania 1971. Environmental Fate of NTA. Proc. 26th Industrial Wastewater Conference, Purdue University, Lafayette, Indiana. 1971. Engineering Extension Series Part 1, p. 271-283.

Forsberg, C. & G. Lindquist 1967. Experimental studies on bacterial degradation of NTA. Vatten 23: 265-277.

Gledhill, W. E. and P. D. McDonald 1976. Microbial Degradation of Builder M and NTA in Laboratory Model Marine and Estuary Systems. Monsanto Internal Report -Interim Report #8448, Sept. 28, 1976. (Cited in Monsanto/Procter & Gamble Report to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders, Cincinnati, Ohio, 1977).

Gregor, C. D. 1972. Solubilization of lead in lake and reservoir sediments by NTA. Env. Sci. Technol. 6: 278-279.

Gudernatsch, H. 1970. Behavior of NTA in the Sedimentation Process and in Sewage. Gass. Wasserfach. 111: 511-516.

Gudernatsch, H. 1975. Biological Degradation of Heavy Metal Complexes of Nitrilotriacetic Acid in Laboratory Activated Sludge Plants. Gass. Wasserfach. 116: 512-517.

Hamilton, R. D. 1977. Presentation to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders. Cincinnati, Ohio, 1977.

Huber, W. & K. H. Popp 1972. Biological Degradation of Nitrilotriacetate Acid in the Presence of Cadmium Ions. Fette Seifen. Anstr. 74: 166-168.

International Joint Commission. 1977. Presentation by Monsanto/Procter & Gamble Companies to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders, Cincinnati, Ohio.

Klein, S. A. 1974. NTA Removal in Septic Tank and Oxidation Pond Systems. Jour. Water Poll. Contr. Fed. 46: 78-88.

Matheson, D. H. 1977. Presentation to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders, Cincinnati, Ohio.

Patrick, R., T. Bott, R. Larson and C. Rhyne 1976. The Effect of Nitrilotriacetate Acid (NTA) on the Structure and Functioning of Aquatic Communities. Draft Report of Grant R801951, for EPA Env. Res. Lab. Duluth, Minn.



Pfeil, B. H. & G. F. Lee 1968. Biodegradation of Nitrilotriacetic Acid in Aerobic Systems. *Env. Sci. Tech.* 2: 543-546.

Prakash, A. 1976. NTA (nitrilotriacetic acid) - An Ecological Appraisal. National Research Council Assoc. Committee for Environ. Quality, NRCC No. 15023, Dept. Fish. Environ., Water Poll. Contr. Dir., Report EPS-3-WP-76-8.

Rudd, J. W. M. & R. D. Hamilton 1972. Biodegradation of Trisodium Nitrilotriacetate in a Model Aerated Sewage Lagoon. *J. Fish Res. Bd. Canada.* 29: 1203-1208.

Rudd, J. W. M., Townsend, B.E. & Hamilton, R. D. 1973. Discharge of nitrilotriacetate (NTA) from two sewage treatment facilities located in a midcontinental climate. *J. Fish Res. Bd. Canada* 30: 1026-1030.

Shannon, E. E. 1975. Effect of Detergent Formulation on Wastewater Characteristics and Treatment. *J. Water Pollut. Control Fed.* 47: 2371-2383.

Shannon, E. E., P. J. A. Fowlie & R. J. Rush 1974. A Study of Nitrilotriacetic Acid (NTA) Degradation in a Receiving Stream. Technology Development Report No. EPS 4-WP-74-7. Water Pollution Control Directorate. Environmental Protection Service. Environment Canada, Ottawa.

Swisher, R. D., M. R. Crutchfield & D. W. Caldwell 1967. Biodegradation of NTA in Activated Sludge. *Env. Sci. Tech.* 1: 820-827.

Swisher, R. D., T. A. Taulli & E. J. Malec 1973. Biodegradation of NTA Metal Chelates in River Water. Trace Metals and Metal-Organic Interactions in Natural Waters. P.C. Singer, Ed., Ann Arbor Science Publishers, Inc., Ann Arbor, Mich., 1973, Cht. 8. p. 237-263.

Shumate, K. S., J. E. Thompson, J. O. Brookhart & C. L. Dean 1970. NTA Removal by Activated Sludge: Field Study. *J. Water Poll. Cont. Fed.* 42: 631-640.

Tabatabai, M. R. & J. M. Bremner 1975. Decomposition of Nitrilotriacetate (NTA) in Soils. *Soil Biol. Biochem.* 7: 103-106.

Taylor, J. K., W. L. Zielinski, E. J. Maienthal, R. A. Durst & R. W. Burke 1972. Development of Method for NTA Analysis in Raw Water. EPA-R2-72-057. Environmental Protection Agency. 27p.

Thompson, J. E. & J. R. Duthie 1968. The Biodegradability and Treatability of NTA. *J. Water Poll. Contr. Fed.* 40: 306-319.

Tiedje, J. M. & B. B. Mason 1974. Biodegradation of Nitrilotriacetate (NTA) in Soils. *Soil Sci. Soc. Amer. Proc.* 38: 278-282.

Tiedje, J. M., B. B. Mason, C. B. Warren & E. J. Malec 1973. Metabolism of nitrilotriacetate by cells of a *Pseudomonas* species. *Appl. Microbiol.* 25: 811-818.

Walker, A. P. 1975. Ultimate Biodegradation of Nitrilotriacetate in the Presence of Heavy Metals. *Prog. Water Technol.* 7: 555-560.



Warren, C. B. & E. J. Malec 1972. Biodegradation of Nitrilotriacetic Acid and Related Imino and Amino Acids in River Water. Science 176, 277-279.

Warren, C. B. & E. J. Malec 1972. Biodegradation of Nitrilotriacetic Acid and Related Imino and Amino Acids in River Water. Science 176, 277-279.







## BIOLOGICAL EFFECTS OF NTA

Canadian monitoring programs (Matheson, 1977; Brownridge, 1977) found detectable (greater than 10 ppb) concentrations of NTA in some rivers and harbors near sewage treatment plant outfalls, particularly in zones of minimal effluent dilution. Reported concentrations of NTA in sewage effluent were essentially the same during summer and winter (Brownridge, 1977). Similarly, sewage treatment plants may release NTA into receiving waters during periods of upset or overloaded conditions. The NTA concentrations released during such periods may approach treatment plant influent concentrations. Thus aquatic organisms living in receiving waters can be exposed to low concentrations of NTA over long periods of time or to elevated concentrations for shorter periods of time. The possibility of NTA exposure raises a number of questions concerning its toxicological behavior:

1. Are the levels of NTA expected to occur in the environment either acutely or chronically toxic to the biota?
2. Are there sublethal effects which might arise and therefore should be investigated? These could include reproductive, metabolic, histologic, or nutritional changes.
3. Are there particular environmental conditions which might enhance the toxicity of NTA to one or more species? For example, how does its biological behavior relate to temperature and water chemistry?
4. How does NTA affect the toxicity of other trace contaminants such as metals?

The results of toxicity tests should be evaluated in light of these and similar questions regarding environmental safety. Since most bioassays, including those with aquatic organisms, are performed with a single test species in a fixed environment, the results are not easily extrapolated to natural ecosystems. Nevertheless they can provide some reasonable boundaries or limits for the predicted response of organisms to NTA exposure.

### EFFECTS ON AQUATIC ORGANISMS

#### ACUTE TOXICITY

Most of the toxicity testing with NTA has involved acute static or flow-through exposures lasting several days. A variety of fish and macroinvertebrates from both freshwater and marine environments have been used. Table 1 and 2 summarize the principal results for both of these groups. The acute LC50 for NTA-Na is generally greater than 100 mg/l for freshwater fish and inverte-



brates. Rainbow trout and the amphipods Gammarus pseudolimnaeus appear to be the most sensitive of the species tested, each having a 96-hour LC50 of 98 mg/l in soft water. Marine organisms tolerate at least a 10 fold greater concentration of NTA. No important differences in sensitivity between taxa are evident.

These reported LC50 values are much greater than the concentration of NTA likely to be found in receiving waters. For example, Brownridge (1977) reported a mean concentration of 0.046 mg/l NTA-Na below sewage plant effluents in areas using detergent formulations with a weighted average of 15% NTA. The maximum measured concentration of NTA in raw wastewater was 22.9 mg/l. Assuming that up to 30% NTA may be used in detergent formulations, the greatest expected NTA concentrations in untreated wastewater would be approximately 40 mg/l. This value is less than the lowest LC50 reported. Maki (1977) calculated a safety factor of 98 for rainbow trout and amphipods based on acute effect levels and a concentration of 1 mg/l of NTA in water. Thus it is unlikely that NTA itself could cause acute mortality of aquatic organisms in waters receiving treated or untreated domestic waste.

The acute bioassay results raise a number of interesting issues. For example, it is unclear whether NTA is the actual cause of death in acute tests. Because of its low toxicity, large quantities of NTA-Na, exceeding 100 mg/l, must be dissolved in the test water. This raises the alkalinity of the solution (Arthur et al., 1974) and may also raise pH, especially in soft waters (the pH of a 1% aqueous solution of NTA-Na is 11). Thus Flannagan (1971), Sprague (1968), and Arthur et al. (1974) attributed at least some of the observed acute mortality to high pH stress. Arthur et al. (1974) were able to increase the static LC50 for fathead minnows from 225 to 470 mg/l (making it appear 2x less toxic) by adjusting pH to 7.7. At the other extreme, tests using the free NTA acid produce abnormally low pH. The 24 hour "lethal concentration" of NTA-H was found by Jancovic and Mann (1969) to be 260 mg/l for rainbow trout and 400 mg/l for tubificid worms in hard water. However, the pH in these tests was 5.0 and 3.4 respectively. Confusion surrounding the interpretation of such results may be avoided in the future by controlling pH, alkalinity, hardness, and chelate concentration independently.

NTA test results are clearly related to the concentration of divalent cations in solution. At high concentrations NTA is essentially all chelated with Ca and Mg. The complexes themselves appear to have no effect on aquatic organisms. Measurable toxicity occurs when NTA levels exceed the molar equivalence point with Ca + Mg. Eisler et al. (1972) found greater toxicity to mummichog at 15°/00, than at 25°/00 salinity. Similarly Acartia clausi a marine zooplankter, gave a 24-hr LC50 of 550 mg/l at 15°/00, but 2100 mg/l at 30°/00, a reduction in toxicity of almost 4 fold (Tarzwell, 1970). It follows that NTA is more toxic to freshwater organisms, particularly in soft waters. Biesinger et al. (1974) reported an excellent correlation ( $r = 0.95$ ) between total hardness and the LC50 to Daphnia magna exposed for three weeks. The toxicity in Lake Superior water, with a total hardness of 45 mg/l as  $\text{CaCO}_3$ , was reduced 4.5 fold when hardness was artificially increased to 200 mg/l. The toxicity-hardness regression for Daphnia predicts an LC50 of 50.56 mg/l NTA-Na at zero total hardness. This value was not verified experimentally.



It is very unlikely that the use of NTA in detergent formulations would lead to acute mortality of freshwater or marine organisms under normal conditions. Yet there may be a possible exception in the case of softwater lakes and streams receiving acid precipitation. These can have sufficiently low pH and hardness to stress indigenous communities. It is not clear whether the release of NTA or other chelating agent would have an effect on such extremely sensitive ecosystems.

#### CHRONIC TOXICITY

Chronic bioassays extending over one or more generations have been performed with several freshwater fish and invertebrates. The maximum no-effect levels for these species exceed the measured environmental concentrations of NTA (Matheson 1977, Brownridge 1977), by several orders of magnitude. Arthur *et al.* (1974) found survival and reproduction of fathead minnows to be unaffected by 54 mg/l NTA, the highest concentration tested. Amphipods, *Gammarus pseudo-limmaeus*, were somewhat more sensitive, showing reduced survival and reproduction above 19 mg/l. Both chronic tests were performed in soft water at near neutral pH. Arthur *et al.* (1970) found no effect on three generations of pulmonate snail, *Physa integra*, at 64.4 mg/l. However, Flannagan (1973) reported decreased adult growth and fecundity above 25 mg/l for the snail *Helisoma trivolis*. *Daphnia magna* in soft water exhibited a 16% reduction in fecundity at an NTA concentration of 91 mg/l, slightly in excess of the molar equivalence point with calcium (Biesinger *et al.*, 1974). The most resistant of the invertebrates tested was the midge, *Chironomus tentans*, which showed no ill-effects at 3000 mg/l NTA (Flannagan, 1971). No information is available on the mode of action of NTA in cases where growth or reproduction was impaired. We do not know, for example, whether metal-NTA chelates can be absorbed or metabolized by the organism during long term tests. Although NTA deposition has been studied in mammalian bone tissue (cited by Thayer and Kensler, 1973) comparable studies have not been performed on the shell-forming mollusks. The only indirect evidence on the site of NTA activity comes from histopathological studies.

#### HISTOPATHOLOGY

Pathological effects of NTA have been reported only for marine organisms. This is surprising in light of its low toxicity in seawater. Eisler *et al.* (1972) found intestinal lesions in mummichog fish exposed for 168 hours to as little as 1 mg/l. Striped bass and scup showed no damage at that concentration. However, bass and mummichog both exhibited damage to the proximal kidney tubules at levels in excess of 3000 mg/l. Grass shrimp exposed to 100 mg/l were found to have pathological changes in intestinal diverticula and kidney. These changes appear to be associated with altered calcium metabolism. Eisler *et al.* (1972) concluded that the damage resembles that seen in isolated kidney tissue maintained in Ca deficient media, and also the kidney damage in dogs dosed with EDTA. Since the work of Eisler *et al.* represents the most serious biological effects of NTA reported to date, it would be highly desirable to repeat and expand on this line of research.



No pathological damage has been detected in freshwater organisms exposed to NTA. Macek and Sturm (1968) observed no gill damage in bluegills exposed to 98 mg/l for 28 days. Kidneys and intestines evidently were not examined. Similarly, Swisher (1968) found no gill changes in bluegill sunfish exposed to the effluent from an activated sludge unit which was fed 200 mg/l NTA along with peptone and LAS. Hamilton (R. D. Hamilton, Freshwater Institute, Winnipeg, unpublished) exposed rainbow trout to as much as 400 mg/l NTA for 100 days. No behavioral or histopathological signs were found.

At this point it is difficult to say whether metabolic and pathological changes are unique to the marine organisms. Calcium metabolism has not been evaluated directly in any of the tests. Furthermore, no chronic studies of NTA absorption or metabolism have been performed on aquatic species. Hamilton (1977) has expressed the belief that NTA does not penetrate gill epithelium, but that the chelating agent draws the metals out of body fluids. Marine fish may have an additional route of exposure not shared by freshwater forms - ingestion and perhaps absorption through the gut. This could partially explain the intestinal lesions in mummichog. Further studies on the mode of action of NTA would be useful in determining its effect on other species under various environmental conditions.

#### METAL INTERACTIONS

There is clear evidence that NTA reduces the toxicity of most divalent metals in solution. Protection against copper and zinc toxicity has been demonstrated with fish (Sprague, 1968; Shaw and Brown, 1974; Gudernatsch, 1970; Kimmerle, 1974; Chynoweth et al., 1976) and with Daphnia magna (Biesinger et al., Kimmerle, 1974; Glass, 1977). Chynoweth et al. have detected the presence of stable Cu-NTA complexes in solution by anodic stripping voltammetry, and an inverse relationship between Cu binding and its toxicity. In the presence of 10 mg/l NTA-Na the 96-hour copper LC50 for juvenile guppies was increased from 0.112 mg/l to 0.224 mg/l. Equimolar concentrations of NTA have also been shown to reduce the toxicity of Cd, Pb, and Ni (Kimmerle, 1974; Eisler et al., 1972; Glass, 1977). However, conflicting results have been noted for  $Hg^{++}$  in the presence of NTA. Eisler et al. reported decreased toxicity of Hg to mummichog. Kania and Beyers (1974) found that 10 mg/l NTA reduced the uptake rate of mercury by Gambusia initially, but increased it somewhat after 18 hours. Glass (1977) found no reduction in chronic Hg toxicity to Daphnia exposed to 0.5 mg/l NTA. Apparently Hg is completely bound by chloride and hydroxide, rather than to NTA. Likewise iron (III) toxicity did not change in the presence of NTA (Biesinger et al., 1974) perhaps because of hydroxide formation.

Concern has often been expressed that chelating agents such as NTA may mobilize toxic metals from sediments, making them more available for uptake by biota. In a series of static and flow-through tests Barica et al. (1973) exposed chironomids, crayfish, and rainbow trout to clean and metal contaminated sediments. When NTA was added at 0.2-5 mg/l, the Fe, Mn, Pb and Zn concentrations in water tended to increase. However the NTA had no significant effect on metal uptake by the organisms. Of all the metals, only Mn showed a slight increase in trout tissue after eight weeks. Chynoweth et al. (1976) found no significant correlation between NTA binding and Cu uptake by fish (even though



toxicity was reduced). Canadian monitoring programs (Brownridge, 1977; Matheson, 1977) have concluded that metal mobilization by NTA is negligible. The Procter and Gamble study (Brownridge 1977), demonstrate that in most cases metals exceeded the molar concentration of NTA in receiving waters. Thus, it is reasonable to conclude that (1) the NTA will not enhance the bioaccumulation of metals from sediment or water, and (2) in most cases it will be present at too low a concentration to afford much protection if metal toxicity occurs.

## TERRESTRIAL ORGANISMS

NTA may find its way onto land if present in appreciable quantities in irrigation water or in sewage sludge applied to soils. Since degradation under anaerobic conditions is slow, NTA may persist in sludges at the time of disposal. However, once on land the NTA is likely to degrade. It therefore appears unlikely that NTA would be present in amounts large enough to pose a hazard to terrestrial plants or animals.

Terrestrial animals would be exposed to NTA primarily through ingestion of residues in food or water. Extensive acute and chronic studies reviewed by Mottola (1974) and Thayer and Kensler (1973) have shown that mammals are tolerant of large oral doses. For example, dogs and rats showed no ill effects when fed 100 mg/kg and 2000 mg/kg respectively, for 90 days. NTA is readily absorbed from the gastrointestinal tract of rats and dogs but not of rabbits or monkeys (cited by Thayer and Kensler, 1973). Virtually all of the NTA absorbed by rats is excreted, unmetabolized, within a few days of ingestion. Thus residue buildup and cumulative toxicity are unlikely in wildlife exposed to the levels expected in the environment.

Land animals may also be exposed to NTA in combination with toxic metals if sludges or dredge spoils contaminated with high concentrations of metals are applied to land. However, synergistic action between metals and NTA is not expected. Rat feeding studies (Thayer and Kensler, 1973) have shown that NTA does not enhance either the uptake or toxicity of ingested methylmercury or cadmium. In fact some of the reported work shows that NTA reduces the effects of metal poisoning.

The influence of NTA on the absorption of metals by higher plants is unclear. Abdulla and Smith (1963) found that NTA increased metal uptake by plants, particularly those growing on alkaline soil. Wallace *et al.* (1974) found no such increase, even at high rates of NTA application. Thus the indirect effect on herbivores of enhanced metal levels in plant tissue cannot be fully evaluated at this time.

## COMMUNITY EFFECTS

The toxicity of NTA has been determined in controlled experiments using a wide variety of test organisms. Several terrestrial and aquatic species have been exposed chronically to constant levels of NTA under laboratory culture conditions. In some cases, the interaction of NTA with other toxicants has also been reported. Thus it is possible to estimate the direct effects of NTA on particular species in the environment. However, few studies have gone beyond the single species approach to evaluate effects at the community or ecosystem level. Factors such as competition, predation, disease, and fluctuat-



TABLE 1

## ACUTE TOXICITY OF NTA-Na TO FRESHWATER ORGANISMS

<u>Species</u>	<u>Effect Measured</u>	<u>NTA-Na Effect Level</u>	<u>Hardness as mg/l CaCO<sub>3</sub></u>	<u>Reference</u>
Rainbow trout, <u>Salmo gairdneri</u>	96-hr LC50	98	35	Macek & Sturm 1973
Fathead minnow, <u>Pimephales promelas</u>	96-hr LC50	127	35	Macek & Sturm 1973
Fathead minnow, <u>Pimephales promelas</u>	96-hr LC50	114	ca. 45	Arthur et al. 1974
Fathead minnow, <u>Pimephales promelas</u>	28 d survival	>96<173	35	Macek & Sturm 1973
Bluegill sunfish, <u>Lepomis macrochirus</u>	96-hr LC50	252	60	Weaver, 1970
	96-hr LC50	487	170	Weaver, 1970
Bluegill sunfish <u>Lepomis macrochirus</u>	28 d survival	>96<173	35	Macek & Sturm 1973
Snail, <u>Physa heterostropha</u>	96-hr LC50	373	60	Weaver, 1970
	96-hr LC50	522	170	Weaver, 1970
Amphipod <u>Gammarus pseudolimnaeus</u>	96-hr LC50	98	ca. 45	Arthur et al. 1974
Amphipod spp.	96-hr survival	≥500	?	Flannagan, 1971
Gastropod spp.	96-hr survival	≥500	?	Flannagan, 1971
11 insect spp. including midge, caddisfly, stonefly, magfly, dragonfly	96-hr survival	≥500	?	Flannagan, 1971
Snail <u>Helisoma trivolvis</u>	96-hr survival	≥100	?	Flannagan, 1974



TABLE 2

## ACUTE TOXICITY OF NTA-Na TO MARINE ORGANISMS

<u>Species</u>	<u>Effects Measured</u>	<u>NTA-Na Effect Level</u>	<u>Reference</u>
Scup, <u>Stenotomus chrysops</u>	168-hr LC50	2200	Eisler <u>et al.</u> 1972
Mummichog, <u>Fundulus heteroclitus</u>	168-hr LC50	5500	Eisler <u>et al.</u> 1972
Striped bass, <u>Morone saxatilis</u>	168-hr LC50	5500	Eisler <u>et al.</u> 1972
Grass shrimp, <u>Palaemonetes vulgaris</u>	168-hr LC50	1800	Eisler <u>et al.</u> 1972
Quahaug clam, <u>Mercenaria mercenaria</u>	168-hr LC50	>10,000	Eisler <u>et al.</u> 1972
Hermit crab, <u>Pagurus longicarpus</u>	168-hr LC50	1875	Eisler <u>et al.</u> 1972
Starfish, <u>Asterias forbesi</u>	168-hr LC50	3000	Eisler <u>et al.</u> 1972
American lobster, <u>Homarus americanus</u>	168-hr LC50	3150	Eisler <u>et al.</u> 1972
Bay mussel, <u>Mytilus edulis</u>	168-hr LC50	3400	Eisler <u>et al.</u> 1972
Eastern mud snail, <u>Nassarius obsoletus</u>	168-hr LC50	5100	Eisler <u>et al.</u> 1972
Sand worm, <u>Nereis virens</u>	168-hr LC50	5500	Eisler <u>et al.</u> 1972
Zooplankton:			
<u>Tisbe furcata</u>	72-hr LC50	270	Tarzwel, 1970
<u>Pseudodiaptomus coronatus</u>	72-hr LC50	700	Tarzwel, 1970
<u>Acartia clausi</u>	72-hr LC50	1350	Tarzwel, 1970
<u>Eurytemora affinis</u>	72-hr LC50	1250	Tarzwel, 1970
Crab zoea	72-hr LC50	1650	Tarzwel, 1970
<u>Tigriopus japonicus</u>	72-hr LC50	3200	Tarzwel, 1970



ing environmental conditions produce stresses which may enhance chronic toxicity. On the other hand, rapid degradation may preclude the possibility of toxic exposures to organisms. Many of these concerns could be addressed by using large scale model ecosystems or field applications of NTA in the presence of other known wastewater constituents. Ecological monitoring in regions currently using NTA in detergents would also be informative.

## REFERENCES

Abdulla, I., and M. Smith, 1963. Influence of Chelating Agents on the Concentration of Some Nutrients for Plants Growing in Soil Under Acid or Under Alkaline Conditions. *J. Sci. F. Agric.* 14: 98-109.

Arthur, A. W., A. E. Lemke, V. R. Mattson, and B. J. Halligan, 1974. Toxicity of Sodium Nitrilotriacetate (NTA) to the Fathead Minnow and an Amphipod in Soft Water. *Water Res.* 8 (3): 187-193.

Barcia, J., M. P. Stainton, and A. L. Hamilton, 1973. Mobilization of Some Metals in Water and Animal Tissue by NTA, EDTA, and TPP. *Water Res.* 7: 1791-1804.

Biesinger, K. E., R. W. Andrew and J. W. Arthur, 1974. Chronic Toxicity of NTA (Nitrilotriacetic Acid) and Metal-NTA Complexes to *Daphnia magna*. *J. Fish. Res. Board Can.* 31 (4): 486-490.

Brownridge, F. A., 1977. Concentrations of NTA in Canadian Waters. In: Presentations Prepared by Monsanto and Procter and Gamble Companies for the IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders.

Chynoweth, D. P., J. A. Black, and K. H. Mancy, 1976. Effects of Organic Pollutants on Copper Toxicity to Fish. In: Toxicity to Biota of Metal Ions in Natural Water, R. W. Andrews, P. V. Hodson, and D. E. Konasewich, eds., International Joint Comm., pp. 145-157.

Eisler, R., G. R. Gardner, R. J. Hennekey, G. LaRoche, D. F. Walsh and P. P. Yevich, 1972. Acute Toxicology of Sodium Nitrilotriacetic Acid (NTA) and NTA-Containing Detergents to Marine Organisms. *Water Res.* 6: 1009-1027.

Flannagan, J. F., 1974. Influence of Trisodium Nitrilotriacetate on the Mortality, Growth, and Fecundity of the Freshwater Snail *Helisoma Trivolvis* through four generations. *J. Fish Res. Board Can.* 31 (2): 155-161.

Flannagan, J. F., 1971. Toxicity Evaluation of NTA to Selected Aquatic Invertebrates and Amphibians. *J. Fish. Res. Board Can. Technical Report* No. 258.

Glass, E., 1977. Identification and Distribution of Inorganic Components in Water: What to Measure? Presented at the Conference on Aquatic Pollutants and Biological Effects with Emphasis on Neoplasia, September 27-29, 1976. The New York Academy of Sciences.

Gudernatsch, H., 1970. Verhalten von Nitrilotriessigsäure im Klarprozess und in Abwasser. *Gas-WasserFach.* 111: 511-516.



- Hamilton, R. D., 1977. Communications to IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders, Chicago, Illinois.
- Jancovic, M. and H. Mann, 1969. Untersuchungen Uber Die Akute Toxische Wirkung Von Nitrilotriessigsäure (NTA). Archiv. Fischereiwiss 20 (2,3): 178-181.
- Kania, H. J., and R. J. Beyers, 1974. NTA and Mercury in Artificial Stream Systems. Ecological Research Series, EPA-660/3-73/025, Environmental Protection Agency. 25 p.
- Kimerle, R. A., 1974. Effect of NTA on Toxicity of Metals and Accumulation of Cadmium in Daphnia Magna and Fathead Minnows. Presented at ASLO Meeting, June, 1974.
- Macek, K. J. and R. N. Sturm, 1973. Survival and Gill Histology of Fish Continuously Exposed to Sodium Nitrilotriacetate (NTA) for 28 days. J. Fish. Res. Board Can. 30 (2): 323-325.
- Maki, A. W., 1977. Aquatic Toxicology of NTA. In: Presentations Prepared by Monsanto and Procter and Gamble Companies for the IJC Task Force on Ecological Effects of Non-Phosphate Builders.
- Matheson, D. W., 1977. Nitrilotriacetic Acid (NTA) in the Canadian Environment. Scientific Series No. 74, Environment Canada.
- Mottola, H. A., 1974. Nitrilotriacetic Acid as a Chelating Agent: Applications, Toxicology, and Bio-environmental Impact, Toxicol. and Environ. Chem. Rev. 2: 99-161.
- Shaw, T. L. and V. M. Brown, 1974. The Toxicity of Some Forms of Copper to Rainbow Trout. Water Res. 8: 377-382.
- Sprague, J. B., 1968. Promising Anti-pollutant: Chelating Agent NTA Protects Fish from Copper and Zinc. Nature 220: 1345-1346.
- Swisher, R. D., 1968. Histological Effects of NTA and Its Degradation Products on Bluegill Fingerlings. Report to Monsanto Company, 1/68 Made Available at the CCIW Meeting, 5/5/70.
- Tarzwel, C. M., 1970. An Evaluation of the Toxicity of Nitrilotriacetic Acid to Marine Organisms. National Marine Water Quality Lab., W. Kingston, R. I., manuscript. 72 pp.
- Thayer, P. S., and C. J. Kensler, 1973. Current Status of the Environmental and Human Safety Aspects of Nitrilotriacetic Acid (NTA), CRC Critical Reviews in Environmental Control, Sept., pp. 357-404.
- Wallace, A., R. T. Mueller, and G. V. Alexander, 1974. Effects of High Levels of NTA on Metal Uptake by Plants Grown in Soil. Agron. J. 66: 707-708.
- Weaver, J. E., 1970. Acute Effects of Sodium Nitrilotriacetate on Fish, Snails, and Diatoms. Procter & Gamble manuscript, 7 pp.







## EUTROPHICATION BY NTA

In its original sense eutrophication referred to an enrichment with plant nutrients. If one of the added nutrients were limiting to the system in question then, according to Liebig's law of the minimum, growth of the plants--algae or weeds--would be stimulated. Modern usage of the term eutrophication retains this usage, indeed the current evaluation of detergent builders stems from the fact that phosphate frequently is a limiting element to algae in lakes, but it goes beyond mere stimulation of algal or weed growth. Today what is generally referred to as eutrophication is the whole complex of changes, chemical, biological, and physical, that results when nutrients are introduced into aquatic ecosystems. In fact, it is often changes other than the increased growth of algae and weeds that cause the greatest concern. For example, in introducing phosphorus to a lake deficient in it, the increased algal growth might not be considered detrimental if it were confined to green algae and/or diatoms, but generally the problem arises because blue-green algae come to predominate. Thus factors leading to a change in composition of the algal communities, as well as those causing an increased abundance of algae, might be considered as causing eutrophication. With regard to the latter it should also be recognized that factors affecting higher trophic levels might result in "apparent eutrophication", e.g. if herbivores were rendered less abundant, algal populations would increase. Therefore, in considering the eutrophication potential of NTA all such possibilities must be considered.

### NTA AS A PLANT NUTRIENT

Sodium NTA contains 5.1% nitrogen, 26.2% carbon, and 25.1% sodium. Although several years ago there was a controversy regarding the importance of carbon as a primary nutrient in aquatic ecosystems (Kuentzel, 1969) today it is generally recognized that carbon-limited systems are rare, and occur only where extreme eutrophication resulting from inputs of phosphorus and nitrogen exists already (Schindler, 1974). Thus, there should be little fear regarding the NTA-caused increase in wastewater carbon. Nitrogen on the other hand can be regarded as a primary nutrient because, although certain algae and bacteria are able to fix atmospheric  $N_2$ , most algae and macrophytes cannot, and must receive it as an inorganic compound--generally as  $NO_3$  or  $NH_3$ . NTA degrades during treatment and it is, therefore, of interest to determine the extent to which NTA-N will contribute to the pool of inorganic nitrogen available for plant growth. The Canadian experience monitored by Procter and Gamble (Brownridge, 1977) showed that 6% NTA detergent resulted in a median concentration of 1.7 mg/l of NTA in waste water, with a maximum of 11.0 mg/l; and that 15% NTA in detergent resulted in a median value of 3.6 mg/l NTA with a maximum of 22.9 mg/l. As these values show fair proportionality



it is likely that 30% NTA in detergent, the greatest amount likely to be present, would give a median wastewater influent value of about 6 mg/l and a maximum concentration of about 40 mg/l. In the Gloucester CFS Detergent Substitute Studies (Shannon and Kamp, 1973) where only NTA detergent was used, influent wastewater NTA averaged 2.19 mg/l, and ranged up to 14.8 mg/l. As the NTA was measured as NTA-H, as NTA-Na it would be 2.9 mg/l and 19.5 mg/l. The detergent used had an NTA-H concentration of 19.7%, i.e. 26% as NTA-Na. Thus, if 30% NTA-Na had been used the average influent concentration would likely have been 3.4 mg/l and the maximum 23 mg/l. These figures are below those of 6 and 40 cited above, so the latter are probably high estimates. Nonetheless, even if these high estimates are used, the nitrogen contribution to wastewater by NTA, assuming complete degradation, would be a maximum of 2 mg N/l, with a median value of about 0.3 mg N/l.

The inorganic nitrogen content of wastewater effluents is generally in the range of 30-40 mg/l. Therefore, on this basis, the maximum increase in N would be 5-6.6%, and the median increase would be 0.75 to 1%.

Nitrogen sources to natural waters are many, including nitrogen fixation, atmospheric precipitation, and land runoff, along with domestic wastes, so that the effect of addition of NTA-N to any given aquatic system would be reduced proportionately. Assuming, for example, that as much as half the nitrogen input to a lake were to come from domestic wastewater, the NTA contribution would then amount to less than 3% of the total at a maximum, and probably less than 0.5%.

Very few lakes appear to be nitrogen-limited, Lake Tahoe being a notable exception, so this danger from NTA appears not great. (Many more lakes have been described to be nitrogen-limited but most of these are so because of the high inputs of phosphorus. Substitution of NTA for phosphorus would help make many of the lakes no longer controlled by nitrogen). Similarly, although marine waters are nitrogen-limited an increase in wastewater nitrogen of the magnitude noted would have at most an insignificant eutrophication effect.

## EFFECTS OF NTA ON ALGAE

Many different approaches have been taken to determine the effects of NTA on algae and to predict the consequences of the use of NTA. NTA has been tested at low concentrations (Goldman and Fujita, 1970); at high concentrations (Weaver, 1970); alone (Erickson, *et al.*, 1970); in the presence of wastes (Sturm and Payne, 1973); against single species of algae in synthetic media (Christie, 1970); in filtered lake water (Weiss, 1970); in microcosms (Swisher and Mitchell, 1970); small enclosures (Bürge, 1974) and large enclosures (Bartsch, *et al.*, 1970); for short periods of time (Goldman and Fujita, 1970); and for long periods of time (Landner, 1969). A variety of criteria have been used to judge the results, ranging from numbers of algae (Sturm and Payne, 1973) through carbon-14 fixation rates (Goldman and Fujita, 1970) to species diversity (Swisher and Mitchell, 1970). To put it simply the results have been variable. For example, Swisher and Mitchell could not find a significant effect of NTA until they used 500 mg/l, but Goldman reported stimulation of carbon-14 uptake at the remarkably low concentration of 0.5 micrograms/l.



In general, the results do demonstrate that NTA cannot be counted on to be stimulatory but that on occasion it may appear to be spectacularly so. It also seems to be clear that NTA itself is not a toxic substance for algae at concentrations likely to be encountered in natural systems, nor is it a stimulant by itself, i.e. in artificial systems. There is also abundant evidence that its positive effects when they do occur are not the result of the increased concentrations of inorganic nitrogen afforded by degradation of the NTA.

In short it looks as though much of the variability in the results derives from the variability of the systems in which the NTA has been tested. This has been made clear in acute toxicity studies on animal organisms where the toxicity is an inverse function of the water hardness (Arthur *et al.*, 1970). But the relationships are more subtle in the case of the algae. It would seem that the variability results from the fact that (a) different systems differ in trace metal content, (b) different systems differ in their community structure.

With regard to trace metals most investigators seem to be aware of the chelating properties of NTA, and many do relate their results to this property, and attempt to explain them in this fashion, but with notable lack of evidence or detail, e.g. Burgi, Goldman. However, there is merit in the proposition. Organisms do live in a milieu in which they are under positive and negative influences. Positive influences include having sufficient nutrients and trace metals for growth. Negative influences include the presence of organic toxins, antibiotics, and detrimental heavy metals. If, for example, photosynthesis by an alga is being inhibited by copper then chelating the copper is likely to stimulate photosynthesis. Depending on circumstances then, NTA could stimulate or inhibit algal populations, and this might be the explanation for some of the variability.

However, it is one thing to discuss reasons for the variability of the results and another to consider the relevancy of the results to the problem at hand. It would appear that most of the results are in fact not relevant. That is, those studies using high concentrations of NTA, or artificial media, or single species of algae, or short periods of observation, while interesting in that they provide clues, cannot tell us what will happen if NTA is released to the natural environment. Only experiments in the natural environment with all its components, and for long enough for the system to respond, can be useful in this regard. That is to say, aquatic systems are ecosystems, not just habitats for algae but systems having various trophic levels. The abundance and types of algae may rest as strongly or even more strongly on the types and abundances of higher trophic levels than on the amount of nutrients. Similarly, the types of algae in a system will depend upon competition between the various species of algae, and this may be subtle. We believe now that many factors can affect these competitive relationships. For example, lowering pH will shift a mixed population of greens and blue-greens toward the greens (Shapiro, 1973). Increasing the N/P ratio may do the same (Schindler, 1977). So may additions of chlorine (Shapiro *et al.*, 1977). On the other hand, increasing the content of magnesium may foster blue-greens (Shapiro, unpubl.), as might the addition of iron. (Murphy *et al.*, 1977) have suggested that blue-green algae excrete hydroxamates that chelate iron and prevent it



from being used by green algae. Addition of a chelator such as NTA could change this relationship but we would not know it unless both types of algae were present. It would appear, therefore, that the most relevant work would be that in which in situ enclosure studies were done for long periods with natural populations. Several exist and are summarized below.

(1) EPA-SHAGAWA LAKE (Bartsch et al., 1970)

Hundred-liter plastic bags of either Shagawa Lake water or Burntside River water were incubated for 13-32 days with various combinations of N, P, secondary and tertiary wastewaters, and NTA, the last at 4-5 milligrams/l. Evaluation was by chlorophyll a.

<u>Treatment</u>	<u>Results</u>
In Burntside water, the results were as follows:	
NTA alone	<u>3</u> experiments + up to 150%; <u>2</u> experiments slightly -
NTA + P	<u>5</u> experiments + up to 225% vs P alone
NTA + tertiary wastewater	<u>1</u> experiment + ca 70% and <u>2</u> slightly - vs tertiary alone
NTA + secondary wastewater	<u>2</u> experiments + ca 30% <u>1</u> slightly - vs secondary alone

In Shagawa water, the results were as follows:

NTA alone	<u>1</u> experiment + 20%; <u>3</u> experiments 0 or slightly -
NTA + P	<u>3</u> experiments + 25-300%; <u>1</u> experiment - 20% vs P alone
NTA + tertiary wastewater	<u>3</u> experiments + 30-100% vs tertiary alone
NTA + secondary wastewater	<u>3</u> experiments + 25-130% vs secondary alone

Thus, in a substantial portion of the cases, NTA did cause stimulation. Examination showed that the effect did not result from the nitrogen in the NTA, as in some cases little degradation had occurred.

(2) EPA-CLINES POND (Bartsch et al., 1970)

Two bottomless enclosures 4.5 x 4.5 x 3 m deep were installed in this eutrophic pond. Five mg/l NTA were added to one and nothing to the other. Observation was for 3 months. The results showed that the NTA treated pond retained much greater algal populations (Anabaena) during July and August. In fact on August 10, at peak phytoplankton production, the maximum rate of carbon fixation in the NTA enclosure was 280 mg C/m<sup>3</sup>/hr. compared with only 30 mg C/m<sup>3</sup>/hr. in the control enclosure. Other parameters such as chlorophyll, transparency, etc. were in accord. Analysis showed that even though all NTA had disappeared by 60 days, the period of heaviest algal growth preceded the period of rapid NTA decrease.



Sizable populations of microcrustaceans were present in both enclosures.

(3) FISHERIES RESEARCH BOARD (Hamilton, 1972)

Twenty, one-m diameter, plastic tubes containing 7,000 liters each were suspended in each of two lakes. NTA with and without sewage at concentrations of 2 micrograms/l to 2 mg/l were used. Monitoring was by many parameters for a whole summer. The results showed no apparent effects of NTA according to Hamilton, although the data have not been published.

(4) BÜRGI (1974)

Plastic bags 1 m x 10 were placed in mesotrophic Lake Lucerne and eutrophic Greifensee (Switzerland). NTA alone, or in combination with iron was added to some bags. Concentrations of NTA used ranged upwards from 50 micrograms/l. In Lake Lucerne significant stimulation by NTA alone occurred, with Oscillatoria rubescens, a blue-green alga; with Cyclotella and Erkenia, diatoms; with Ankistrodesmus and Chlorella, greens; and with Cryptomonas and Rhodomonas, cryptophytes. The diatom Tabellaria and the green Mougeotia were inhibited. In Greifensee most species were inhibited by the NTA additions.

(5) PATRICK ET AL.--PHILADELPHIA ACADEMY (1976)

The effects of various concentrations of NTA up to 200 milligrams/l were tested in small stream systems run through a laboratory for several months. No effect was noted on bacterial activity but algal shifts were seen, e.g. Spirogyra was more abundant at 2 mg/l NTA and there was a shift to blue-greens at 200 mg/l, but the total biomass was much reduced.

(6) SWISHER AND MITCHELL--MONSANTO (1970)

Microcosms containing one liter of mud and seven liters of lake water and reinoculated every two weeks with lake water were maintained for periods of up to two years. NTA was added with and without sewage at concentrations of from 2 mg/l to 500 mg/l. Total organic carbon and diversity index of the algal populations were used as the criteria for effect. The only effect noted was at 500 mg/l NTA where "excessive bloom conditions" were induced.

(7) LANDNER (1969)

During 3 summer months in 1969 a study was made in Sweden on a system of lakes and streams receiving the discharge from a wastewater treatment plant treating an influent containing 4-6 mg/l of NTA.

The plant consisted of a trickling filter, a sedimentation pond, and an oxidation pond. The mean effluent volume was 1500 m<sup>3</sup>/day. A number of physico-chemical parameters, along with the occurrence and abundance of different phyto- and zooplankton organisms as well as bottom organisms, were measured at several stations upstream and downstream of the outfall. No trace of NTA was found in the water system during the period. Landner



believes that none of the effects observed on the biota could be unequivocally ascribed to the discharge of NTA. He gives it as his opinion that because eutrophic water systems already have a large amount of naturally chelating material, addition of artificial chelators would be of little consequence.

As with the other experiments the results of these seven were variable. Stimulation was found by the EPA using Cline's Pond, Shagawa Lake, and Burntside River water, and algal changes were found by Bürgi and Patrick, and by Swisher and Mitchell, while the Swedish study and the FRB study were negative. However, none of these studies really satisfies all criteria. For example, the EPA studies used unrealistically high concentrations of NTA. No algal composition data are given for them, and although zooplankton were present in the Cline's Pond system with NTA, their specific nature is not described. Were they daphnids, which could explain the low algal population in the control? Were the zooplankters in the control system the same? Similarly, no data are available to us from the FRB studies. Was there a shift in algal species or in zooplankton composition? Bürgi's study is good in that species composition was carefully examined, but unfortunately the study was too short. Also the reasons for the species changes were not clear--were they caused by zooplankton? Patrick's study was restricted to a very few members of the stream ecosystem. For example, no herbivores were present. The Swedish study is difficult to interpret as the effect of the sewage itself is very great. Finally, it has not been possible to find studies of the effects of NTA on macrophytes, or on the attached alga, Cladophora, which is a great nuisance in the inshore waters of the Great Lakes, exactly where concentrations of NTA would be greatest. The only near relevant paper is one by Stockner and Evans (1974) in which they report little effect of NTA on attached algal communities.

#### REFERENCES

- Arthur, J. W., K. E. Biesinger; A. E. Lemke; R. W. Andrew; V. R. Mattson  
1970. Direct Toxicity and Some Interaction Properties of Nitrilotriacetic Acid (NTA) to Aquatic Life. Mimeo report, National Water Quality Laboratory, Duluth.
- Bartsch, A. R. and 15 other authors, 1970. Field and Laboratory Investigations to Evaluate the Influence of Nitrilotriacetic Acid (NTA) on Eutrophication. May-November, 1970. Unpub. Report to Commissioner, Federal Water Quality Administration.
- Brownridge, F. A., 1977. Report for the IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders.
- Bürgi, H. R., 1974. Die Wirkung von NTA auf das Wachstum des Phytoplanktons unter besonderer Berücksichtigung des Eisens als Mikroelement. Schweiz. Zeit. f. Hydrol. 36: 1-70.
- Christie, A. E., 1970. Trisodium Nitrilotriacetate and Algae. Water and Sewage Works, 1970. pp. 58-59.



Erickson, S. J.; T. E. Maloney and J. H. Gentile, 1970. Effect of Nitrilotriacetic Acid on the Growth and Metabolism of Estuarine Phytoplankton. J. Water Poll. Contr. Fed. 42: R329-325.

Goldman, C. R. and D. Fujita, 1970. The Response of Naturally Occurring Phytoplankton Populations to NTA in Lake Tahoe, Castle Lake and Clear Lake, California. In: Bartsch et al., 1970.

Hamilton, R. D. 1972. The Environmental Acceptability of NTA - Current Research and Areas of Concern. In: "Nutrients and Eutrophication". Special Symposia, Vol. 1. ASLO. Ed. G. E. Likens.

Kuentzel, L. E., 1969. Bacteria, Carbon Dioxide, and Algal Blooms. J. Water Poll. Contr. Fed. 41: 1737

Landner, L., 1969, Kartläggning Av Ett Recipientsystem Med Särskild Hänsyn Till Utsläpp Av NTA-Haltigt Kommunalt Avloppsvatten. Mimeo Report, Swedish IVL, Stockholm, December 1969.

Murphy, T. P.; D. R. S. Lean and C. Nalewajko, 1976. Blue-Green Algae: Their Execution of Iron-Selective Chelators Enables Them to Dominate Other Algae. Sci. 192: 900-902.

Patrick, R.; T. Bott, R. Larson, and C. Rhyne, 1976. The Effect of Nitrilotriacetic Acid (NTA) on the Structure and Functioning of Aquatic Communities. Draft Report of Grant R801951 for EPA Env. Res. Lab. Duluth, Minnesota.

Schindler, D. W., 1974. Eutrophication and Recovery in Experimental Lakes: Implications for Lake Management. Science 184: 897-899.

Schindler, D. W., 1977. Evolution of Phosphorus Limitation in Lakes. Science 195: 260-262.

Shannon, E. E. and L. J. Kamp, 1973. Detergent Substitution Studies at C.F.S. Gloucester. Tech. Dev. Rept. # EPS 4-WP-73-3. Water Pollution Control Directorate. EPS, Environment Canada, Ottawa.

Shapiro, J., 1973. Blue-Green Algae: Why They Become Dominant. Science 179: 382-384.

Shapiro, J.; G. Zoto, and V. Lamarra, 1977. Experimental Studies on Changing Algal Populations From Blue-Greens to Greens, Unpub. Manuscript.

Stockner, J. G. and D. Evans, 1974. Field and Laboratory Studies on the Effect of Nitrogen, Phosphorus and NTA Additions on Attached Algal Communities. Fish. Res. Bd. Canada Tech. Report No. 416. 109 pp.

Sturm, R. N. and A. G. Payne, 1973. Environmental Testing of Trisodium Nitrilotriacetate: Bioassays for Aquatic Safety and Algal Stimulation. In: G. E. Glass, (Ed.). Bioassay Techniques and Environmental Chemistry: 403-424. Ann Arbor, Science Publishers, Inc.



Swisher, R. D. and D. T. Mitchell, 1970. NTA and the Environment. A Review of the Literature and Work in Progress. Internal Report, Inorganic Chemicals Division, Monsanto Co.

Weaver, J. E., 1970. Acute Effects of Sodium Nitrilotriacetate on Fish, Snails, and Diatoms. Manuscript submitted for publication. June 1970.

Weiss, C. M., 1970. Influence of Nitrilotriacetate (NTA) on Eutrophication. In Situ and Batch Assays. In: Bartsch et al. 1970.



## MODELLING OF NTA CONCENTRATIONS IN WATER

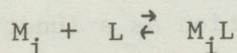
Little work has been done on the modelling of NTA (or any other organic ligand) concentrations in natural waters. A number of attempts have been made to describe the equilibrium distribution of NTA and its complexes based on thermodynamic aspects (Childs 1971, Chance 1975, Cilley and Nicholson 1971, Winters 1977), but only Lerman and Childs (1973) have attempted to integrate all factors governing expected levels in water into a predictive model. This paucity of attempts is undoubtedly due to the inherent complexity of a model requiring consideration of not only thermodynamic relationships determining complex formation but also rates of breakdown for both the ligand and each of its complexes. Because the breakdown of NTA and its complexes involves complex reactions that have as yet been quantified only in a few very specific cases (eg. Shannon, Fowlie and Rush 1974), a realistic, usable model has yet to be defined and tested. The approach of Lerman and Childs is certainly the most complete employed to date and is outlined and commented on below.

The concentration of NTA in natural waters will depend on 3 factors:

- 1) thermodynamic relationships between NTA, other organic ligands, and metals, including adsorption-desorption phenomena. The equilibrium distribution of NTA between "free" material and each complex will depend on the chemical composition of the water in question,
- 2) the rate of degradation of NTA and each of its complexes,
- 3) the water replenishment rate of the body of water (which may be modified according to its thermal structure), the loss of material by sedimentation, and the input rate to the body of water.

As stated above, the thermodynamic relationships governing distribution of NTA between its various chemical species have been summarized by several authors.

Each metal ion ( $M_i$ ) may undergo complex formation with a ligand L:



with equilibrium constant  $K_i = [M_iL]/[M_i][L]$ .



The metal-ligand complex concentration is then -

$$[M_i L] = K_i [M_i] f [L_T]$$

where  $L_T$  is the total concentration of the ligand (in this case, NTA) and  $f$  is a fraction defined for each given ionic composition. If the concentration of the organic ligand is small relative to that of the various metal ions, the  $[L_T]$  can be defined as:

$$L_T = [L] \left( 1 + \sum_{i=1}^n K_i [M_i] \right)$$

If however,  $[L]$  is large, then

$$L_T = [L] \left( 1 + \sum_{i=1}^n \frac{K_i ([M_i] + [M_i L])}{1 + K_i [L]} \right)$$

Solution of a series of such equations is possible (by computer) if metal concentrations, ligand concentrations (all ligands, not just NTA), and all equilibrium constants are known. The aforementioned references, in effect, do just that using a defined data set. The equilibrium distribution of NTA in water of a specified chemical composition can therefore be approximated. However, the use of this technique is limited by the fact that most organic ligands in natural water are uncharacterized. Their equilibrium constants are therefore unknown, and the magnitude of the effects on the distribution of NTA among its various forms is uncertain.

The degradation of NTA and its complexes may occur because of bacterial, oxidative or photochemical processes (for more details see previous sections). If, as a first approximation, it is assumed that decomposition occurs without equilibrium; that is, without continuous readjustment between the ligand's complexes, then the total rate of decay of the ligand is:

$$- \frac{d[L_T]}{dt} + \frac{d[M_1 L]}{dt} \dots + \frac{d[M_{n-1} L]}{dt} + \frac{d[L]}{dt}$$

where  $-\frac{d[M_i L]}{dt} = \lambda_i [M_i L]$  and  $\lambda_i$  is the first-order reaction rate constant.

Therefore, the total NTA concentration as a function of time is given by:

$$[L_T] = [L_T(o)] f_1 e^{-\lambda_1 t} + \dots f_m e^{-\lambda_n t}$$



if  $[M_i L]$  at  $t = 0$  is a fraction,  $f_i$  of  $L_T$ .

If, however, there is continuous re-equilibration, then the total concentration of NTA is given by:

$$\begin{aligned} [L_T] &= [L_T(0)] e^{-(f_1 \lambda_1 + f_n \lambda_n)t} \\ &= [L_T(0)] e^{-\Lambda t} \end{aligned}$$

In this case, total decomposition occurs at a faster rate.

Other factors that will determine the NTA concentration in a body of water are the input rate ( $J$ ) of the NTA, flushing rate ( $\rho$ ) of the body of water, and the rate of loss to sediments of the NTA. If the body of water is assumed to be instantaneously and homogeneously mixed and if the interaction of NTA with sediments can be described by the Freundlich absorption equation ( $C_s = KC^n$ , where  $C_s$  is the concentration of NTA on the substrate,  $K$  is a constant,  $C$  is the concentration of NTA in the water, and  $0 < n < 1$ ), then the rate of change of the concentration of NTA in the body of water can be described by:

$$\frac{dC}{dt} = J - \lambda C - \rho C - \frac{dC_s}{dt}$$

where  $\lambda$  is taken as a decay constant indicative of NTA and all its complexes. The solution to this equation is given by Lerman and Childs, both for the case where the sediment interaction is described by the Freundlich equation and for the simpler case where there is no interaction (ie.  $dC_s/dt = 0$ ). In the latter instance, a steady-state concentration of NTA  $C_{ss}$  will be reached more slowly; nevertheless, the actual concentration attained will be the same whether or not the sediment interaction is taken into account. It is given by:

$$C_{ss} = J/(\rho + \lambda)$$

Clearly, the assumption that a single decay constant ( $\lambda$ ) can be used to describe the decomposition of all NTA complexes oversimplifies the situation. If, however, this is accepted as a very crude approximation, then estimates of steady-state NTA concentrations of water bodies of various flushing rates can be made based on specific values of  $\lambda$ . For example, if the "combined" half-life for decomposition of NTA and all its complexes in a given body of water is 2 weeks and the flushing rate =  $1 \text{ yr}^{-1}$ , then the steady-state concentration will be 5.3% of the input concentration.

A more realistic model might incorporate  $n$  simultaneous equations:

$$\frac{dC_i}{dt} = J - \lambda_i C_i - \rho C_i - \frac{dC_{s,i}}{dt} \quad \text{where } i = 1 \text{ to } n$$



with continuous thermodynamic equilibrium between the  $n$  complexes (including "free" NTA). The decomposition rates,  $\lambda_i$ , are not only unique but are functions of temperature, etc. While such a model is easy to formulate, the data required to use it do not exist.

To set upper limits for NTA concentrations expected, one could assume decomposition rates are zero ( $\lambda_i = 0$ ) and that there are no sediment interactions  $\frac{dC_s}{dt} = 0$ . The concentration is, therefore, governed by

input dilution only. Evidence exists, however, to show that decomposition rates are relatively rapid (ie. loss by decomposition will be greater than loss by washout for almost all bodies of water); thus, this simple approach will result in unrealistically high estimated NTA concentrations.

In summary, although it is possible to formulate mathematically a model that approximates the expected behaviour of NTA in natural waters, the data required to employ an even crude simplification of it are so extensive as to be prohibitive.

## REFERENCES

- Chance, J.M. 1975. Computer Simulation Studies on the Environmental Effects of Nitrilotriacetic Acid. M.A. Thesis, Univ. of Pennsylvania, Penn., 72 p.
- Childs, C.W. 1971. Chemical Equilibrium Models for Lake Water which Contains Nitrilotriacetate and for "Normal" Lake Water. Proc. 14th Conf. Great Lakes Res.:198-210.
- Cilley, W.A. and D.A. Nicholson. 1971. An Equilibrium Model for the NTA Complexation of Metal Ions in Natural Waters. Env. Letters 2:121-129.
- Lerman, A. and C.W. Childs. 1973. Metal Organic Complexes in Natural Waters: Control of Distribution of Thermodynamic, Kinetic and Physical Factors. p.201-235. In P.C. Singer (ed.). Trace Metals and Metal-Organic Interactions in Natural Waters. Ann Arbor Science Publ. Inc., Ann Arbor, Michigan.
- Shannon, E.E., P.J.A. Fowlie and R.J. Rush. 1974. A study of Nitrilotriacetic Acid (NTA) Degradation in a Receiving Stream. Report No. EPS 4-WP-74-7. 25p.
- Winters, R.E. 1977. Chemistry of NTA. 26p. In Presentation Prepared by Monsanto and Procter & Gamble Companies for the IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders. Cincinnati, Ohio.



## ENVIRONMENTAL LEVELS OF NTA

### WASTE TREATMENT PLANTS

The calculated average concentration of NTA expected in raw municipal wastewater has ranged between  $3.2 \text{ mg l}^{-1}$  (Brownridge 1977) and  $75 \text{ mg l}^{-1}$  (Epstein 1972). A realistic concentration for the post-1973 period when detergents in Canada had an average content of 15% NTA, is  $5 \text{ mg l}^{-1}$  (Matheson 1976, Can. Dept. Nat. Health Welfare 1972), with levels expected to reach  $10 \text{ mg l}^{-1}$  if all laundry STPP were replaced by NTA. Concentrations in household wastes in rural areas employing septic tank-tile field or holding tank systems, may reach 2 to 3 times that in municipal wastewater (Can. Dept. Nat. Health Welfare 1972), resulting in levels of up to  $20\text{--}30 \text{ mg l}^{-1}$ .

Measured influent concentrations are summarized in Tables 1 (from Thayer and Kensler 1973) and 3 (from Toetz 1977), although in many of the studies reported, NTA was added to the influent to achieve a desired concentration. Effluent concentrations which are also summarized in Tables 1 and 2, were highly variable ( $0.01 - 11.7 \text{ mg l}^{-1}$ ) and depend on many factors, e.g. type of treatment, influent concentration, temperature etc., as previously discussed.

### DOMESTIC WATER SUPPLIES

Two major programs designed to monitor NTA concentrations in drinking water have been undertaken. The first was carried out in Suffolk and Norfolk Counties, New York, in the winters of 1970 and 1971, and is summarized by Thayer and Kensler (1973). Of 279 samples taken from 129 households, six had detectable NTA levels of between  $25$  and  $125 \text{ } \mu\text{g l}^{-1}$ , three of these from well water supplies, and three from public utilities.

The second study, which was much more widespread, was carried out in Canada by Procter and Gamble and the Canada Department of the Environment from October 1971 to 1975, and included sampling of wastewater treatment plant influents and effluents, rivers, streams and lakes, and groundwater, as well as domestic supplies. Results have been summarized in detail by Matheson (1976) and Brownridge (1977) and will be only briefly outlined here.

The drinking water supplies of 13 towns and small cities (population  $1,900\text{--}64,000$ ) in southern Ontario were sampled monthly for 30 months. The concentration of NTA was less than  $60 \text{ } \mu\text{g l}^{-1}$  in all samples, and most (96%) contained less than  $25 \text{ } \mu\text{g l}^{-1}$  (Table 3). Metal levels (Cu, Cr, Cd, Pb, Zn, Ni, Al, Fe, Na) were also monitored, and while levels were low, no measurements were made prior to NTA usage.

In addition, surveys of drinking water in other parts of Canada have been carried out and results are summarized in Table 4. A total of 698



samples were analyzed, of which 33 had NTA concentrations between 10 and 80  $\mu\text{g l}^{-1}$ .

## LAKES AND RIVERS

Some of the lakes and rivers surveyed for NTA as part of the Canadian monitoring program are included in the discussion of Domestic Water Supplies (above) as they served as domestic water supplies for towns or cities. Other special cases (Hamilton Harbour, western Lake Ontario, St. Lawrence River) are discussed in Special Studies (below). In addition, river water upstream and downstream from 13 towns and cities in southern Ontario was monitored by Procter and Gamble (Brownridge 1977) for 39 months. Median concentrations in the streams above the wastewater outfalls of the cities ranged from 3 to 28  $\mu\text{g l}^{-1}$  in the period when NTA made up 6% of detergent composition, and from < 1 to 38  $\mu\text{g l}^{-1}$  when detergents averaged 15% NTA. In general, medians were not higher in the latter period. Below the wastewater outfalls, medians ranged from 3 to 61  $\mu\text{g l}^{-1}$  at 6% NTA and 1 to 123  $\mu\text{g l}^{-1}$  at the 15% level, with 10 of 13 locations showing increases in the second period. Of these 3 were significantly different at the 95% probability level.

## GROUNDWATER

A discussion of the degradation of NTA in groundwater is given in an earlier section, and a summary of NTA levels in groundwater used for domestic water supplies in Canada has been provided in the discussion of Domestic Water Supplies (above). Only 1 of 78 samples had a detectable concentration (50  $\mu\text{g l}^{-1}$ ), and only on one occasion was this site found to be contaminated.

Furthermore, of the 13 towns whose drinking waters were extensively studied by Procter and Gamble, six used groundwater as a source. Results are summarized in Table 3.

In addition, other special studies have been carried out to determine NTA concentrations under conditions most likely to lead to contamination of groundwater (Matheson, 1977):

- a) Finch, Ontario - 21 of 68 wells situated in shallow overburden (10-14 feet of soil on bedrock) had 15 to 250  $\mu\text{g l}^{-1}$  NTA. Some very high values were subsequently found (up to 1.7 mg  $\text{l}^{-1}$ , but high concentrations coincided with bacterial contamination.
- b) Stonewall, Manitoba - 1 of 28 wells was contaminated with NTA (290  $\mu\text{g l}^{-1}$ ).
- c) Brandon, Manitoba - 4 of 6 wells had measurable NTA concentrations (13-82  $\mu\text{g l}^{-1}$ ), all again showing evidence of sanitary pollution.

In summary, NTA levels in groundwater may be substantial, particularly in cases where contamination by domestic sewage is evident. Small, privately constructed wells are more likely to be contaminated than municipal groundwater sources.



## SPECIAL STUDIES

Three areas of special interest have been studied in Canada as part of the national monitoring program (summarized by Matheson 1976):

- a) Hamilton Harbour - the relatively small ( $21 \text{ km}^2$ ,  $2.8 \times 10^8 \text{ m}^3$ ), isolated western-most portion of Lake Ontario receives wastewater effluent from population of 400,000. During the time when Hamilton wastewater received only primary treatment, the mean NTA concentration of the effluent was  $0.8 \text{ mg l}^{-1}$  which was subsequently reduced to  $0.25 \text{ mg l}^{-1}$  after secondary treatment came into effect. Monthly monitoring of seven stations from December 1971 to February 1975 allowed the following important conclusions to be drawn:
  - 1) the concentration in the harbour did not change significantly after a reduction in the input rate;
  - 2) the concentrations in winter were no higher than those in summer;
  - 3) average concentration at six stations remote from the outfall was  $15 \text{ } \mu\text{g l}^{-1}$ ; nearer the outfall it was  $60 \text{ } \mu\text{g l}^{-1}$ ; and
  - 4) degradation of NTA must have occurred in the harbour itself as well as in the treatment plant; the concentration in the harbour was, in fact, about 12% of that expected, based on dilution of plant effluent alone;
- b) Western Lake Ontario - Seven open water stations were sampled 14 times in 1972 and 1973 in western Lake Ontario. Positive values ( $10\text{-}160 \text{ } \mu\text{g l}^{-1}$ ) were found at several locations in the first few months but not thereafter. Drinking water from ten municipal waterworks with intakes near shore had  $< 10 \text{ } \mu\text{g l}^{-1}$  in 38 of 40 samples, the highest value being  $20 \text{ } \mu\text{g l}^{-1}$ .
- c) St. Lawrence River - Monitoring of the St. Lawrence River between Lake Ontario and Montreal in three months of 1973 produced no detectable levels of NTA. In the metropolitan Montreal area, however, samples on occasion contained up to  $30 \text{ } \mu\text{g l}^{-1}$ . Distribution of positive samples could be related to local discharges. Maximum concentrations below Montreal were  $10 \text{ } \mu\text{g l}^{-1}$ . Matheson (1976) calculated the entire undegraded NTA usage of metropolitan Montreal would result in a level of only  $30 \text{ } \mu\text{g l}^{-1}$  by dilution alone; thus, the results of this survey are not surprising.

## REFERENCES

Brownridge, F.A. 1977. Report for the IJC Task Force on Ecological Effects of Non-Phosphate Detergent Builders.



Can. Dept. Nat. Health Welfare. 1972. Review of Toxicity and Potential Biological Effects of NTA. Dept. of National Health and Welfare, Ottawa, 283 pp.

Epstein, S.S. 1972. Toxicological and Environmental Implications on the Use of Nitrilotriacetic Acid as a Detergent Builder - I. Int. J. Environ. Studies 2: 291-300.

Matheson, D.H. 1977. Nitrilotriacetic acid (NTA) in the Canadian Environment. Scientific Series No. 74. Env. Can.

Thayer, P.S. and C.J. Kensler. 1973. Current Status of the Environmental and Human Safety Aspects of Nitrilotriacetic Acid (NTA). CRC Critical Rev. in Env. Control 3: 375-404.

Toetz, D. W. 1977. Fate, Transport and Effects of NTA in the Aquatic Environment. Draft report, EPA.



TABLE 1  
NTA CONCENTRATION IN TREATMENT PLANT INFLUENTS AND EFFLUENTS  
(from Thayer and Kensler 1973)

System	Study	NTA Concentration (ppm)	
		Influent	Effluent
Trickling filter	Sweden	2-7	0.2-2.2
	Iowa	4, 8, 16	0-1.6
Activated Sludge	Sweden	5	2
	Wisconsin	40-118	<2
	Ohio	8	1.5
	Ohio	16	4 (av.)
	Virginia	11-79	<1
	Laboratory	20	1-2.5
	Laboratory	30	0<3
	Laboratory	10 (continuous)	<1



TABLE 2  
REMOVAL OF NTA BY WASTEWATER TREATMENT SYSTEMS (from Toetz) 1977

Source	Type of Treatment	Location	NTA Concentration, mg/l			% of Removal
			Influent	Effluent	Receiving Stream	
Thayer & Kensler (1973)	Primary	Canada	0.8-3.3	ND	0.02-0.04	10.9-30.1
Thayer & Kensler (1973)	Activated Sludge	Canada	2.8-5.0	0.01-1.75	0.02-0.18	21.5-82.5
Shannon, et al. (1974)	Activated Sludge	Canada	8-20	0.3-0.9 (summer) 5.4-11.7 (winter)	0-0.01 0.03-0.11	95 45
Shannon (1975)	Activated Sludge	Canada	0.67 2.19	0.18 0.86	ND	73.1 60.6
Shumate, et al. (1970)	Activated Sludge	Ohio	2 8 16*	0.2 0.8 4.0	ND	89.4 90.0 75.2
Renn (1974)	Activated Sludge	Virginia	40-50*	0.3-1.0	ND	80-90
Klein (1974)	Septic Tank	California	30-60*	ND	ND	21.8-23.1
Klein (1974)	Aerobic laterals	California	60*	0.6-3.0	ND	95-99
Klein (1974)	Anaerobic laterals less/0.5 mg/l DO	California	60*	45	ND	10
Cleasby, et al. (1974)	Trickling filter	Iowa	4-8*	0.5-2.0	ND	82-100
Thayer & Kensler (1973)	Trickling filter	Canada	1.3-4.5	0.9-2.9	0.01-0.80	21.6-65
Klein (1974)	Oxidation ponds	California	2-30* 75* 100*	0.2-3.0 15 50	ND ND ND	90 80 50
IJC (1976)	Primary	Canada	0.01-23	0.01-7.67	0.005-0.063	--
	Activated Sludge	Canada	0.01-23	0.03-8.75	0.004-0.912	--
	Trickling filter	Canada	0.01-23	1.35-10.5	0.003-0.103	--
	None	Canada	0.01-23	ND	0.00-0.01	--

\*NTA dosed into influent to give stated levels

NOTE: ND = No data



TABLE 3  
NTA CONCENTRATION IN DRINKING WATER IN THIRTEEN CITIES IN SOUTHERN ONTARIO  
(from Brownridge 1977)

Frequency Distribution of NTA Concentrations in Drinking Waters

NTA Concentrations $\mu\text{g}/\ell$	% of Samples having less than Stated Value		
	Rivers	Wells	Lakes
1	24	59	34
2	32	72	43
5	55	88	78
10	75	97	98
15	84	99	100
25	92	99.5	
40	97	100	
60	100		



TABLE 4

NTA LEVELS MEASURED IN DRINKING WATER SUPPLIES IN CANADA  
(data from Matheson 1976)

Location	No. of Samples with Detectable Levels of NTA	Source of Water Supply
British Columbia	1 sample of 126 ( $60 \mu\text{g l}^{-1}$ )	Campbell River
Alberta	2 of 26 ( $10-20 \mu\text{g l}^{-1}$ )	S. Saskatchewan River
Saskatchewan	4 of 26 ( $10-20 \mu\text{g l}^{-1}$ )	ivers
Manitoba	1 of 28 ( $20 \mu\text{g l}^{-1}$ )	Burntwood River
Ontario	5 of 180	Great Lakes - St. Lawrence River - Ottawa River
	5 of 32	small rivers
	0 of 22	small lakes
	1 of 51	groundwater
	1 of 18 ( $10 \mu\text{g l}^{-1}$ )	Richelieu River
Quebec	5 of 12 ( $20-80 \mu\text{g l}^{-1}$ )	Yamasha River
	4 of 60 ( $10-20 \mu\text{g l}^{-1}$ )	small lakes
	4 of 50 ( $20-30 \mu\text{g l}^{-1}$ )	small rivers
	0 of 80	groundwater
Maritimes	0 of 59	



## APPENDIX

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### Subjects

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TABLE 4

DATA LAID DOWN IN DRINKING WATER SUPPLIES IN CANADA  
(Data from Statistics Canada 1976)

Location	Level of Drinking Water Supply	Source of Water Supply
Alberta	10-15 (10-15)	Campbell River
British Columbia	10-15 (10-15)	S. Saskatchewan River
Manitoba	10-15 (10-15)	river
Ontario	10-15 (10-15)	Burnwood River
Quebec	10-15 (10-15)	Great Lakes
		St. Lawrence River
		Ottawa River
		small rivers
		small lakes
		groundwater
	10-15 (10-15)	Richelieu River
	10-15 (10-15)	Yamaska River
	10-15 (10-15)	small lakes
	10-15 (10-15)	small rivers
	10-15 (10-15)	groundwater



FIRST MEETING  
OF THE  
GREAT LAKES RESEARCH ADVISORY BOARD'S  
TASK FORCE ON  
ECOLOGICAL EFFECTS OF NON-PHOSPHATE DETERGENT BUILDERS

Held at the Airport Hilton Hotel (Dorval)  
Montreal, Quebec  
December 6, 1976

Members

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C. R. O'Melia	University of North Carolina

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F. Kennedy	Continental Oil Company

Representing Federal Governments

W. Lowe	Canada Centre for Inland Waters
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Secretary

D. R. Rosenberger	International Joint Commission, Great Lakes Regional Office
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SECOND MEETING  
OF THE  
RESEARCH ADVISORY BOARD'S  
TASK FORCE ON  
ECOLOGICAL EFFECTS ON NON-PHOSPHATE DETERGENT BUILDERS

Held at the Americana Inn  
Cincinnati, Ohio  
February 8-9, 1977

Members

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P. Dillon	Ontario Ministry of Environment
G. Leduc	Concordia University
R. Dick	University of Delaware
P. J. Chapman	University of Minnesota
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THIRD MEETING

OF THE

GREAT LAKES RESEARCH ADVISORY BOARD'S

TASK FORCE ON

ECOLOGICAL EFFECTS OF NON-PHOSPHATE DETERGENT BUILDERS

Held at the  
Sheraton O'Hare Hotel  
Chicago, Illinois  
February 21-22, 1977

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FOURTH MEETING  
OF THE  
RESEARCH ADVISORY BOARD'S  
TASK FORCE ON  
ECOLOGICAL EFFECTS ON NON-PHOSPHATE DETERGENT BUILDERS

Held at the Cambridge Motor Inn  
Toronto, Ontario  
March 14, 1977

Members

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G. Leduc	Concordia University
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Secretary

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FIFTH MEETING  
OF THE  
RESEARCH ADVISORY BOARD'S  
TASK FORCE ON  
ECOLOGICAL EFFECTS OF NON-PHOSPHATE DETERGENT BUILDERS

Held at the IJC  
Great Lakes Regional Office  
April 19, 1978

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## TERMS OF REFERENCE

### INTERNATIONAL JOINT COMMISSION - RESEARCH ADVISORY BOARD

#### TASK FORCE ON ECOLOGICAL EFFECTS OF NON-PHOSPHATE DETERGENT BUILDERS

Eutrophication of the Great Lakes remains one of the serious problems to which the Great Lakes Water Quality Agreement is addressed. Phosphorus has been acknowledged to be the nutrient limiting algal growth and, for this reason, programs to control the input of phosphorus are presented in Annex 2 of the Agreement.

A significant proportion of the phosphorus entering the Great Lakes is due to phosphates discharged from municipal sewage treatment plants. Therefore, the Agreement specifies that waste treatment facilities shall be constructed and operated to remove phosphorus from municipal sewage.

The Annual Report of the Water Quality Board and the Final Report of the Upper Lakes Reference Group both presented at the July 1976 meeting of the International Joint Commission addressed the questions of phosphorus discharge from municipal wastewater treatment plants. As a significant proportion of the phosphorus in sewage arises from detergent usage both reports recommend limitations on the phosphorus content of detergents.

As such a ban or limitation on phosphates in detergents will require alternative builder compounds and/or levels to be utilized, relevant ecological information regarding the effects of such materials must be gathered and interpreted to permit the Commission to evaluate the potential consequences of such detergent reformulation based upon the best available scientific information. To provide this information the Research Advisory Board should review the information on ecological effects of non-phosphate detergent builders in present use.

To provide this information the Task Force will:

1. Review and summarize the research findings on detergent builder alternatives to phosphate.
2. Identify areas of research in which there are gaps in our knowledge relative to the effects of potential alternate builder materials.
3. Report to the Research Advisory Board on the adequacy of studies pertaining to these materials and identify both these materials which, based on present information, are judged to be ecologically acceptable and those which are not.



The Task Force will consider:

1. Expected discharge levels and the extent to which ambient concentration will be increased.
2. Effects on the sewage treatment process.
3. Biodegradation and physicochemical transformations.
4. Expected or known degradation products with special reference to the more stable ones.
5. Toxicity to biota.
6. Chelation of trace metals and mobilization of metals.

When used in appreciable quantities, all types of detergent builders can potentially effect the ecosystem of the Great Lakes. Upon completion of its review of the ecological effects of non-phosphate detergent builders, the Task Force will compare the types and extent of impacts expected with the use of non-phosphate builders to that of phosphorus.

#### MEMBERSHIP

Task Force members should include an aquatic biologist, an aquatic toxicologist, an environmental chemist, a modeler, and a waste treatment engineer. To provide necessary liaison with the industry a non-voting member should represent the U.S. and the Canadian Soap and Detergent Associations. Persons selected to serve on the Task Force should be knowledgeable regarding both the open literature and the report literature. To provide the most adequate evaluation and report, scientific information input should be solicited from both government and industry.



## MEMBERSHIP LIST

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